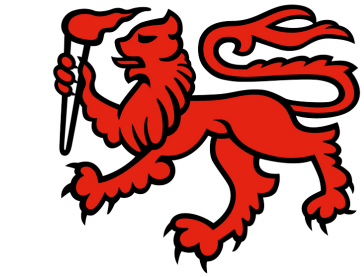

Dynamic resilience and stability of *Ecklonia radiata*: the importance of density-dependent ecosystem engineering feedbacks

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This thesis is dedicated to all the volunteers who assisted me in the field and in the lab. Without their contribution, it would not have been possible to complete this research.

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Statement of co-author contributions

Chapter 2-4 of this thesis have been prepared as manuscripts for submission to peer-reviewed journals. The conducts of the research (experiment designs, method developments, analysis, interpretation of results, manuscript preparation) were responsibility of the candidate, but were consulted with supervisors and other co-authors. These contributions are outlined below.

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Jeffrey Wright and Craig Johnson contributed to the conception and design of the experiment, and analysis and interpretation of the results. Cayne Layton and Matthew Cameron contributed to development of methodologies and conduct of filed work along with Victor Shelamoff. All co-authors provided comments on the manuscript.

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Abstract

Kelps are the dominant marine ecosystem engineers of temperate subtidal rocky reefs around the globe. Kelp forests create a complex habitat structure, modify abiotic factors and support diverse and highly productive systems. However, kelps face several threats and degradation of kelp forests has been reported in a number of places including south-eastern Australia. To date, most studies of threats to kelps focus on how external factors contribute to the degradation but there are also important internal drivers that will also influence kelp stability and resilience. Because ecosystem engineers modify the abiotic and biotic environment, the changes they make may positively feedback to affect their own demography. Consequently, a decline in adult density may lead to a decline in engineering capacity, a change in the modified conditions and reduction in, or loss, of positive effects.

Ecklonia radiata is the most widespread habitat-forming kelp in Australia, however, range of stressors, such as ocean warming, more severe and frequent storms, overgrazing and pollution have been impacting its populations causing localised declines in population density. Although studies have been conducted to understand the implications of these changes, how a decline in adult density will affect the demography (reproduction, recruitment, post-recruitment growth and survivorship) of *E. radiata* are poorly understood. The central aims of this thesis were to determine; (i) how a decline in the density of *Ecklonia* affects engineering of critical abiotic factors and the link between those abiotic changes and both the understory community and *Ecklonia* reproduction, recruitment and post-recruitment growth and survivorship, (ii) whether abiotic factors modified by *E. radiata* were important mechanisms affecting the early post-recruitment survivorship and growth of *E. radiata* sporophytes, and; (iii) how density-dependent effects on reproductive output affected recruitment of both gametophytes and sporophytes.

Chapter 2 presents a study where I manipulated the density of adult *E. radiata* in a field experiment for 24 months to four different levels (zero, low, medium and high) and measured changes to abiotic factors, associated understory algae and *E. radiata* recruit demography. These manipulations revealed density-dependent engineering of some, but not all abiotic factors. Most notably, light increased as *E. radiata* density decreased; scour increased at low and medium *E. radiata* densities compared to high; while sediment accumulation increased in

the absence of *E. radiata* compared to any treatments with *E. radiata*. Despite some density-dependent changes in abiotic factors, the understory algal community and demography of *E. radiata* recruits did not always reflect these changes due to large variation in these metrics within the density treatments which highlighted the variable nature of these processes acting on small-spatial scales on natural reefs.

In chapter 3, I manipulated three modified abiotic factors (light, scour and water flow) in the field to identity their role as mechanisms affecting the survivorship and growth of early sporophyte recruits. Overall, this experiment revealed low light / low scour (ambient flow) as important for very small / early sporophytes, but the response of slightly larger sporophytes was more complex. By week 6 in the absence of scour, light and water flow interacted and the highest survivorship occurred with ambient flow/low light and low flow/ambient light. Importantly, these larger sporophytes respond positively and grow much faster under higher light.

Chapter 4 tested density-dependent issues related to reproduction. Specifically, how reproductive capacity of *E. radiata* in the field (the amount of zoospores released per individual) was affected by adult *E. radiata* density and how recruitment of microscopic stages (gametophytes and sporophytes) was influenced by zoospore density in two different seasons. Zoospore released per individual did not vary greatly in relation to adult density indicating that the total amount of zoospores produced in a *E. radiata* forest is likely to increase as the number of reproductive adults increases. Gametophyte and sporophyte recruitment were dependent on the density zoospores, with clear optimal ranges (90-355 mm^{-2} in spring and 21-261 mm^{-2} in winter) and threshold densities (< 6.5 mm^{-2} in spring < 0.5 mm^{-2} in winter) of zoospores for successful recruitment.

Overall, this study indicates that some abiotic factors (light, scour and sediment accumulation) change in response to a declining density of *Ecklonia* and that low scour and combinations of light and water flow are important to post-recruitment survivorship and growth. However, the evidence for the break-down of a positive demographic feedback with a decline in *E. radiata* density was not strong. Nonetheless this study did indicate that a high density of reproductive *E. radiata* sporophytes is likely to result in a high density of zoospores and subsequently higher recruitment of microscopic sporophytes beneath the canopy. The sub-canopy conditions beneath a high-density *E. radiata* forest (low light, reduced scour and understory algal abundance) are also likely to result in high survivorship

of microscopic sporophytes. Once light increases due the creation of localised gaps in the canopy, these recruits grow fast allowing the canopy to reform highlighting a key component to the resilience of *E. radiata* forests.

Chapter 1. Introduction

Marine ecosystems around the globe are being exposed to increasing levels of anthropogenic stressors including urban development, climate change, excess nutrients, declining water quality and overfishing (Jackson et al. 2001, Scheffer et al. 2001, Airoldi 2003, Ling 2008, Gribben et al. 2013, Wernberg et al. 2016). Climate change is often considered the most significant threat to marine species, including habitat-forming ecosystem engineers, and cumulative evidence of changes to populations of ecosystem engineers and the species that rely on them for habitat have been reported (Ridgway 2007, Johnson et al. 2011, Kroeker et al. 2013, Wernberg et al. 2016, Provost et al. 2017). The increased frequency and intensity of stressors can degrade key coastal habitat created by ecosystem engineers such as kelp, coral, seagrass and mangroves (Poloczanska et al. 2007, Wernberg et al. 2010, de Fouw et al. 2016, Goatley et al. 2016). As these species are fundamental for the health of these coastal ecosystems, loss of these ecosystem engineers can lead to declines in diversity, simplified and low productive systems and in extreme cases, catastrophic phase shifts (Scheffer et al. 2001, Folke et al. 2004, Krumhansl et al. 2016, Wernberg et al. 2016, Chefaoui et al. 2018).

Ecosystem engineers are species which modify the environment in ways that often enhance productivity and species diversity (Jones et al. 1994, Coleman and Williams 2002, Hastings et al. 2007). Engineer species are categorised as either autogenic or allogenic engineers (Jones et al. 1994). Autogenic engineers modify the environment by simply being present in a system (e.g. kelps providing shades and seagrass reducing sediment transfer rate; Kennelly 1989, van der Heide et al. 2012) while allogenic engineers actively modify the environment through their behaviour (e.g. beavers changing flows of water by creating a dam and pika enhancing soil nutrient levels in arid sytem by digging; Jones et al. 1994, 1997, Godet et al.

2008). Modifications to the environment by ecosystem engineers can occur in three ways: structural, abiotic and biotic engineering (Jones et al. 2010). For autogenic ecosystem engineers, structural engineering involves the insertion of the physical structure of the engineer (for example, a tree or a bivalve shell) into the environment. The structure leads to abiotic change (abiotic engineering: for example, a decrease in light or boundary flow) and both structural and abiotic engineering lead to biological change (biotic engineering) such as changes to community structure (Jones et al. 2010).

Here I present a conceptual framework highlighting how structural, abiotic and biotic engineering in a marine ecosystem engineer such as kelp, can result in a feedback to the engineer itself (also see Jones et al. 2010). The presence of kelp causes structural engineering of the environment, which in kelp forests results in the modification of abiotic factors such as light, sedimentation, scour and water flow (Eckman et al. 1989, Kennelly 1989, Madsen et al. 2001, Toohey et al. 2004, Tatsumi and Wright 2016). Associated biotic communities, such as understory algae and invertebrates, are then determined by the presence of kelp structure and abiotic factors (Connell 2003b, Toohey 2007, Flukes et al. 2014). Kelp recruits are also influenced by the presence of the kelp structure (Anderson et al. 1997, Taylor and Schiel 2005) modifying abiotic factors such as light and sedimentation (Novaczek 1984, Bearham et al. 2013, Geange et al. 2014) and biotic factors such as competition and grazing (Anderson et al. 1997, Tatsumi and Wright 2016). Consequently, changes to the structural, abiotic and biotic environments beneath the kelp canopy can positively affect the demographic rates of kelp including reproduction, recruitment and post-recruitment survivorship. This positive feedback to the demography of an engineer has been termed an environment-engineer feedback (Jones et al. 2010) and may be crucial to the stability and resilience of ecosystem engineering species. Although not all feedbacks will be positive, overall effects should be

positive for populations of the engineer species to remain stable. Additionally, the degree of feedback effects are expected to be density dependent (Hastings et al. 2007, Jones et al. 2010, Wernberg et al. 2016), therefore population degradation which results in a decline in density may affect the engineering capacity and thus the feedback. If there is a threshold density where positive feedbacks are lost, then identifying this threshold may be important in the management of these crucial habitats.

Kelps are important ecosystem engineers of temperate sub-tidal rocky reefs (Steneck et al. 2002, Steneck and Johnson 2014, Krumhansl et al. 2016). The abiotic factors they modify can change in density-dependent ways (Gerard 1984, Wernberg et al. 2005) and influence associated sub-canopy benthic communities (Gerard 1984, Dayton et al. 1992, Clark et al. 2004, Tatsumi and Wright 2016). Light in particular is significantly reduced under intact kelp canopies by as much as 90% (Wernberg et al. 2005), reducing the biomass of turfing, filamentous and foliose understory algae with understory communities beneath a full canopy typically dominated by encrusting algae and sessile invertebrates (Connell 2003a, Tatsumi and Wright 2016). Turfing, filamentous and foliose algae are generally more common in canopy gaps where there is more light available (Connell 2003a, Wernberg et al. 2005, Toohey and Kendrick 2008). Similarly, a 66% decline in the kelp canopy causes changes to the understory community, with more foliose algae with fewer sponges, bryozoans and encrusting algae compared to a full canopy (Flukes et al. 2014). As understory algae are direct competitors of kelp recruits (Kennelly 1987, Toohey and Kendrick 2007, Tatsumi and Wright 2016), an increase in foliose understory algae associated with a decline in kelp density may result in conditions that decrease kelp recruitment. To our knowledge, the connection between density-dependent modification of abiotic parameters, biotic community

change and how these affect the demography of engineers such as kelp has not previously been tested.

Ecklonia radiata (herein referred as *Ecklonia*) (C. Agardh) J. Agardh (order Laminariales) is the most wide-spread marine ecosystem engineering alga of Australasia and it dominates much of the temperate sub-tidal coastlines of southern Australia (Womersley 1967, Bennett et al. 2016). *Ecklonia* forests support productive and diverse communities including a number of economically important species (Johnson et al. 2005, Bennett et al. 2016). *Ecklonia* has a typical kelp life-cycle consisting of microscopic male and female gametophytes and a macroscopic sporophyte stage (Womersley 1967, Schiel and Foster 2006). Its maximum height is two meters and consists of a holdfast and a single stipe from which arises a central lamina with paired lateral developments, although large morphological variation has been reported (Womersley 1967, Jennings and Steinberg 1997, Fowler-Walker et al. 2006, Wernberg et al. 2010, Mabin et al. 2013). The reproduction season also varies depending on location with peaks in summer on the west coast but in cooler months on the east coast (Mabin et al. 2013, Mohring et al. 2014). Populations of *Ecklonia* on both the east and west coasts of Australia are increasingly being impacted by the southwards movement of warm water associated with boundary currents which negatively affects *Ecklonia* directly and indirectly via associated movement of grazers, causing population degradation (Ling and Johnson 2012, Mabin et al. 2013, Wernberg et al. 2016). As positive feedback effects by *Ecklonia* may be density dependent, a reduction in density may have implication for the stability and resilience of populations.

When considering factors affecting the population degradation of habitat-forming kelps, most previous research has focussed on external drivers, such as increased ocean temperature, severe storms and over grazing (Seymour et al. 1989, Ling and Johnson 2012, Wernberg et al. 2013, Filbee-Dexter et al. 2016, Vergés et al. 2016) with less attention on internal drivers such as a decline in a positive environment-engineer feedback from adult sporophytes. Although *Ecklonia* is known to modify abiotic and biotic factors (Kennelly 1989, Connell 2003a, Connell 2003b, Wernberg et al. 2005, Flukes et al. 2014), little is known about whether these modifications change in a density-dependent manner, and how this feeds back to influence its recruitment and post-recruitment performance. Survivorship of early life-history stages of kelp is typically low and highly variable (Schiel and Foster 2006, Tatsumi and Wright 2016) Kelp recruits are often observed beneath the adult canopy (Anderson et al. 1997, Wernberg et al. 2010) where they are likely to be influenced by modified sub-canopy environment. Performance of juvenile sporophytes is likely to be influenced by a range of external factors (Ling & Johnson 2012, Wernberg et al. 2016), but engineering modifications by adult sporophytes may reduce stressors, improving recruitment success. However, mechanisms determining the recruitment and post-recruitment growth and survivorship of sporophytes is not well known. In addition, we know little about factors influencing population replenishment in *Ecklonia*, particularly possible density-dependent effects on reproductive output (amount of zoospore released) and how zoospore density influences gametophyte and subsequent sporophyte survivorship and growth. Predicted ocean warming, more frequent and severe storms, grazing and other anthropogenic disturbances are likely to cause further degradation of kelp forests (Ling and Johnson 2012, Filbee-Dexter et al. 2016, Vergés et al. 2016, Wernberg et al. 2016) hence, understanding ecosystem-engineering feedback mechanisms and how these changes in density-dependent manner is critical.

Thesis structure

Much of current research on *Ecklonia* has focused on how external drivers influence its stability and resilience, but internal drivers affecting its demography, environment-engineer feedback in particular, may also be important but are not well ununderstood. To reveal the dynamic nature of density-dependent engineering feedback, helping to define any tipping points where positive engineering effects may be lost, I set the central aims of this thesis to determine: (i) how a decline in the density of *Ecklonia* affected engineering of critical abiotic factors and the link between those abiotic changes and both the understory community and *Ecklonia* reproduction, recruitment and post-recruitment growth and survivorship, (ii) whether abiotic factors modified by *Ecklonia* were important mechanisms affecting the early post-recruitment survivorship and growth of *Ecklonia* sporophytes, and; (iii) how density-dependent effects on reproductive output affected recruitment of both gametophytes and sporophytes. Aims (i) and (ii) were addressed using field experiments and aim (iii) by a laboratory experiment.

Chapter 2: Density-dependent effects of *Ecklonia radiata* on sub-canopy abiotic conditions, understory algae and recruitment.

The density of adult *Ecklonia radiata* was manipulated to four different levels for 24 months to test for density-dependent engineering of abiotic factors, associated biotic communities and effects on *Ecklonia* demographic rates. The demography of both microscopic (by outplanting cultured sporophytes) and macroscopic (by transplanting) juveniles were measured under each density treatment.

Chapter 3: Interactive effects of canopy-driven changes in light, scour and water flow on self-recruitment in kelp.

A field experiment was conducted to determine interactive effects of three key abiotic factors engineered by *Ecklonia*: light, scour and water flow, on the survivorship and growth of microscopic sporophytes. Each abiotic factor had two treatment levels (light: ambient/low, scour: present/absent, water flow: ambient/low) crossed with a second abiotic factor and the effects of these treatments on the survivorship and growth of outplanted cultured sporophytes was determined at two times.

Chapter 4: Density-dependent and seasonal variation in reproductive output, sporophyte production and performance in the kelp, *Ecklonia radiata.*

This chapter combined long-term data on seasonal and density-dependent reproductive output (measured as zoospore release) with a laboratory experiment that determined the effects of variable zoospore density on subsequent recruitment. Density-dependent zoospore release was monitored in the field for 24 months while the effects of zoospore density was examined in the lab using a dilution series experiment.

Chapter 5: General discussion

In the final chapter, I synthesise the overall findings of the research and drawing on ecosystem engineering theory, discuss the implications of my research for the stability and resilience of kelp. I also discuss how my findings can contribute to the management and restoration of *Ecklonia* and highlight possible further avenues of research. My research highlighted some engineering effects of a healthy *Ecklonia* forest in providing a suitable sub-canopy environment for microscopic sporophyte recruitment (low light and low scour) but the effects of a decline in density were inconsistent.

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Chapter 2. Density-dependent effects of *Ecklonia radiata* on sub-canopy abiotic conditions, understory algae and recruitment

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Abstract

Ecosystem engineers, such as kelp, play critical roles in temperate coastal marine systems, modifying biotic and abiotic resources and supporting diverse communities. However, degradation of kelp populations, including a decline in local density, has been observed in a number of places. A decline in kelp density may change the modification of abiotic factors and influence the associated biotic community as well as negatively affect the kelp's own recruitment and post-recruitment growth and survivorship beneath the canopy (i. e. a positive demographic feedback). We manipulated the common kelp, *Ecklonia radiata*, to four difference densities (high [natural], medium, low and zero) and maintained these densities for 24 months. We measured density-dependent changes in: 1) five important abiotic factors potentially engineered by *Ecklonia* (sediment deposition and accumulation, water flow, light and benthic scour), 2) understory algal richness, biomass and community structure, and 3) recruitment and performance (survivorship, growth and erosion) of macroscopic juvenile sporophytes and the survivorship of outplanted microscopic sporophytes. There were density-dependent changes to sub-canopy light (up to 90% increase) and scour (~ 10% increase) in low and medium density treatments compared to high density, and a threshold response for sediment accumulation (increased in the absence of *Ecklonia*) but no density-dependent effects on sediment deposition and water flow. Abiotic factors often showed large temporal and small-scale spatial variation which appears to have resulted in limited density-dependent effects on the understory community, with understory algal biomass only increasing in the absence of *Ecklonia*. Similarly, the recruitment and post-recruitment performance of microscopic and macroscopic recruits was highly variable in time and space with limited evidence of density-dependent effects. Although a high density of kelp creates sub-canopy abiotic conditions that should be suitable for the recruitment of *Ecklonia* (low light, scour and sediment accumulation) the large variation for most metrics highlighted the importance of understanding processes acting at the microscale to more fully understand the existence of a positive demographic feedback in *Ecklonia*.

Introduction

Ecosystem engineer species play critical roles in many systems, modifying biotic and abiotic resources and supporting diverse communities (Jones et al. 1994, Coleman and Williams 2002, Hastings et al. 2007, Jones et al. 2010). Marine autogenic ecosystem engineers include invertebrates (e. g. corals, bivalves, tubeworms) and macrophytes (e. g. mangroves, seagrasses, seaweeds). These species create three-dimensional habitat and structural complexity that causes local environmental change via modification of abiotic factors with large flow-on effects for associated species (Bertness et al. 1999, Bruno et al. 2003, Altieri et al. 2010, van der Heide et al. 2012, Kimbro et al. 2014, Wright and Gribben 2017) as well as for the engineer itself. The complex food webs supported by marine ecosystem engineers are often resilient to external stress (Elmqvist et al. 2003), however, multiple stressors, such as ocean warming, the introduction and migration of species, over fishing, nitrification and other pollution, and increased sedimentation can threaten marine ecosystem engineers (Jackson et al. 2001, Airoldi 2003, Ling 2008, Gribben et al. 2013, Wernberg et al. 2016). These stressors can result in reduced densities of ecosystem engineers (Wernberg et al. 2011, Waser et al. 2015) and thus their capacity to modify key biotic and abiotic parameters.

Abiotic factors which may be modified by marine ecosystem engineers including light, temperature, water flow, water chemistry, sedimentation, substratum stability, and benthic scouring (Kennelly 1989, Wernberg et al. 2005, Altieri et al. 2010, Gribben et al. 2013). The modification of these factors is often density dependent, and a decline in the density of marine macrophytes can result in changes in these factors that can potentially impact associated species that use the engineer as habitat. For example, lower macrophyte densities result in higher light, benthic sediment cover (Wernberg et al. 2005, Toohey 2007), water flow (Madsen et al. 2001), dissolved oxygen, and sediment redox potential (Gribben et al.

2013, Gribben and Wright 2014) beneath the canopy. Higher light typically results in more understory algae and a different understory community (Airoldi 2003, Clark et al. 2004, Toohey and Kendrick 2008, Wernberg and Goldberg 2008, Flukes et al. 2014, Strain et al. 2015), while increased sediment can smother small organisms or early stages of the life-cycle (Geange et al. 2014), and changes in water chemistry can influence the behaviour, growth and survivorship of associated species (Gribben et al. 2013, Gribben and Wright 2014). These abiotic and/or biotic changes can in turn affect the recruitment and performance of the engineer itself. Examples of self-facilitation or positive environment-engineer feedback (*sensu* Jones et al. 2010) include increased survivorship of barnacle recruits at a high density due to reduced temperature (Bertness et al. 1999), greater recruitment of cockles as adult cockle density increases due to increased sediment accumulation (Donadi et al. 2014), and reduced grazing of seagrass as density increases and more sediment accumulates (van der Heide et al. 2012). Despite the potential far-reaching consequences, relatively few studies have experimentally determined density-dependent effects of ecosystem engineers on abiotic factors, associated species and self-facilitation (D'Andrea and DeWitt 2009, Jones et al. 2010).

Kelps are the dominant ecosystem engineers of temperate reefs world-wide and the habitat they create support diverse assemblages of fish and invertebrates (Steneck et al. 2002, Steneck and Johnson 2014, Krumhansl et al. 2016). Kelps engineer abiotic resources through their structure. Their canopies create shade causing a significant reduction of light and, in some cases, increase physical abrasion of the benthos (Wernberg et al. 2005, Toohey and Kendrick 2007). Canopy development can also reduce local water velocity which can affect sub-canopy sediment deposition rate (Eckman et al. 1989, Layton et al. in review). Under a full kelp canopy there is often a low biomass of understory algae but a high cover of

encrusting algae and sessile invertebrates such as sponges and bryozoan (Kennelly 1989). The increase in light and sediment cover with declining kelp canopy (Wernberg et al. 2005) is usually correlated with a shift from an encrusting algal and invertebrate-dominated understory community to a foliose algal-dominated community (Kennelly 1989, Clark et al. 2004, Toohey et al. 2004, Flukes et al. 2014). This dense understory algal canopy has implications for the resilience of kelp forests as they compete with kelp recruits (Kennelly 1987, Geange 2014, Tatsumi and Wright 2016).

Although density-dependent ecosystem engineering may be expected in autogenic engineers such as kelp, studies often focus on presence/absence of the engineer species with limited information on density-dependent modifications of biotic and abiotic factors. Wernberg et al. (2005) demonstrated density-dependent engineering by *Ecklonia radiata* (hereafter referred to as *Ecklonia*) on light and sedimentation but not water flow in a naturally occurring kelp forest, with light and sediment cover decreasing with increasing *Ecklonia* density. In this study, we examine density-dependent engineering of the abiotic environment by *Ecklonia* and the consequences for the associated understory community and its own recruits by manipulating adult *Ecklonia* sporophyte density for 24 months at four different levels: high (natural), medium, low and zero. We determined 1) how density affected five important abiotic parameters engineered by kelp: sediment deposition and accumulation, water flow, light, and benthic scouring, 2) the response of understory algal communities to examine the link between long-term reductions in density, abiotic and biotic change, and 3) how density of adult sporophytes affected the recruitment and the post-recruitment performance of transplanted microscopic and macroscopic recruits of *Ecklonia*.

Materials and methods

Study species

Ecklonia radiata is the most abundant habitat forming ecosystem engineer of the Great Southern Reef of Australia (Bennett et al. 2016). The habitat provided by *Ecklonia* supports diverse and productive food webs including commercially important species. The economic value generated by these reefs is at least AU$10 billion a year from fishing and tourism (Wernberg et al. 2011, Ling and Johnson 2012, Bennett et al. 2016). *Ecklonia* occurs from depths of a few meters down to 50 m (Marzinelli et al. 2015), but dense forests are typically found between ~ 8 to 20 meters deep. Adult sporophytes grow up to two meters tall with large latitudinal and localised morphological variations and forming a thick canopy (Womersley 1967, Mabin et al. 2013).

Study site

The experiment was conducted in Fortescue Bay, on the south-east coast of Tasmania (43° 08' S, 147° 58' E). In the bay, *Ecklonia* forms a monospecific canopy between 8 – 15 m depth at an average density of 9 -12 adult thalli m^{-2} (Flukes et al. 2014). In Feb 2014, we established twelve 5 x 5 m plots at a depth of 11 ± 2 m along approximately 150 m stretch of a reef on the northern side of the bay. Treatments were allocated randomly to the plots as one of four densities of adult *Ecklonia* (high: un-manipulated = 9.3 ± 1.5, medium: 4-5, low: 1-2, and zero: 0 adult *Ecklonia* thalli m^{-2}). Densities were manipulated by removing adult *Ecklonia* (including holdfasts) by hand until the desired density was reached. Although we manipulated *Ecklonia* density in the entire 5 x 5 m plots, we took all our measurements in the central 3 x 3 area of each plot to minimise edge effects from the surrounding continuous forest. Plots were delineated by a rope border (3 m x 3 m) on the benthos which was tied at each corner to heavy weights. As *Ecklonia* continued to recruit into the plots and grow

throughout the experiment, we removed adult *Ecklonia* as required approximately every 3 months for the duration of experiment (24 months) to maintain the treatments.

Sedimentation deposition

In each plot, we measured sediment deposition into sediment traps five times during the first year of the experiment. Sediment traps were constructed from PVC cylinders 300 mm in height and 50 mm in diameter with baffles installed at the entrance to the traps. This height-to-diameter ratio and the baffles minimised resuspension of captured sediment from the traps (Jürg 1996). A frame of galvanised steel was fixed to approximately the centre of each plot to which four traps were attached (two above the *Ecklonia* canopy and two below the canopy). The traps were installed in March, April, June, July and November 2014 and left for approximately 40 days, with the exception of the November deployment which remained in the water for 120 days due to logistical challenges. The collected traps were capped and taken back to the lab and the sediment left for 1-2 days to settle. Excess water was removed, and sediments were flushed into pre-weighed foil trays and dried at 70˚C oven for 48 hours. Any obvious organisms, such as gastropods, in the samples were removed prior to drying. The dried samples were weighed and converted to g sediment m^{-1} day^{-1}. We calculated the percentage increase or decrease in sediment deposition beneath the canopy. If a sediment trap was missing or not suitable to take a measurement (i.e. the top was blocked or the trap was hanging at an angle), only one sediment trap was used. This happened to only three sediment traps during the experiment.

Sediment accumulation

We measured sediment accumulation within the turf sediment matrix seven times over the duration of the experiment. We selected 10 random points in each plot and placed a small

ruler vertically to measure the depth of the turf-sediment matrix (to 1 mm accuracy). Measurements were taken in March, April, June, September, November 2014 and May, December 2015.

Scouring of the benthos by kelp laminae

We measured relative scour by using a wire sensor method similar to that outlined by Beermann et al. (2013). We inserted 49 thin copper wires as a 7 x 7 equidistant grid into a 7 x 4.5 cm kitchen sponge. The sponge had been pre-soaked in casting plaster to make it rigid but still allow the copper wires to be inserted. The sensors were left to dry at room temperature for 24 hours and then dried at 50˚C for a further 24 hours. They were then attached to a 9 x 4.5 cm PVC panel which had heavy duty Velcro attached to the other side. Prior to deployment of the sensors, five pieces of the other side of the Velcro were randomly attached in each of the 12 plots using underwater epoxy (A-788 Splash Zone Compound). Five sensors were then attached in each plot making sure that all wires were at the 90˚ position (vertical) at the start of the experiment so any canopy abrasion on the sensors will deform the copper wires and provide a measure of relative canopy scour. We also deployed five control sensors which were surrounded by a mesh cage to prevent contact with kelp laminae. Understory algae around all panels were removed to isolate the scouring effect of the *Ecklonia* canopy. The sensors were left in place for four days after which the angle that each wire had departed from 90˚ was measured. This experiment was conducted in late February 2016 (at the end of the experiment), during low wave exposure in the bay (Mundy & Keane, unpubl.).

Water flow

Relative water flow among treatments was measured using plaster blocks (i.e. clod-cards). Clod-cards were made by mixing casting plaster ($CaSO_4$) and fresh water as recommended

by the manufacturer (20 :13 plaster : water ratio). The casting plaster was poured into a 500 ml mould containing a 50 mm steel bolt, to enable later attachment in the field. The clod-cards were left for 24 hours and then dried at 50˚C for further 24 hours, labelled, weighed and attached to a PVC base-panel (equipped with a mesh cage to stop scour of the plaster by *Ecklonia* laminae) for installation. In the field, one panel each with one clod-card was attached to the base of the frame supporting the sediment traps (below the canopy) and another one on the top of the frame (above the canopy) as a 'control' to measure ambient flow. One pair of the clod-cards was installed in each of the 12 plots in October and November 2014. Clod-cards were left in the field for 4 to 5 days, retrieved and taken back to the lab. They were removed from the PVC base-panel, dried at 50˚C for 24 hours and weighed. Pre- and post-deployment weights were used to calculated mass loss per day and the percentage difference between clod-cards above and below the canopy was calculated for each pair to determine the effects of different canopy density on water flow beneath the canopy.

Irradiance

Photosynthetic Active Radiation (PAR) was measure in two ways. In November 2014, we set up sites at the same depth but outside the main experimental plots in which we applied the same four adult density treatments in which we measured *in-situ* density-dependent PAR using fixed Odyssey PAR loggers. These plots were approximately 1.5 m^2 in size and we ensured that the canopy outside the plots did not interfere with the measurements. PAR was measured continuously in these plots from Nov 2014 to Aug 2015 with average accumulated PAR recorded every 15 min. Although these measurements provide information on temporal changes in PAR over time, because the Odyssey PAR loggers only measure irradiance from ~ 90˚ directly above the sensor, this may not be an accurate representation of PAR below the

canopy. Consequently, we also used a handheld LI-COR LI-1500 Light Sensor Logger with a LI-193 Spherical Underwater Sensor. Unlike traditional flat sensors, this sensor can detect PAR from ~ 330˚, which allows the measurement of incidental PAR that enters the kelp patches horizontally and thus is a more accurate reflection of irradiance beneath the canopy. The sensor was attached to the end of a 1.8 m pole to avoid shading from divers and measurements taken above and below the canopy for 60 seconds at the centre of each of the twelve 5 x 5 m plots to calculate the percentage of light reduced by the different canopy treatments. PAR was logged automatically every 15 second as mean μmol photon m^{-2} s^{-1} (n = 4). The average of ambient (above canopy) PAR in each plot was used to calculate sub-canopy PAR reduction. The measurements were taken on sunny days between 11:00 to 14:00 in June and Nov 2015.

Species richness, biomass and community structure of understory algae

At the end of experiment (March 2016), we conducted a survey to determine whether different *Ecklonia* densities over 2 years caused changes in understory algal species richness, biomass and community structure. We placed five 0.5 m x 0.5 m quadrats randomly (but avoiding positions used for measuring scour) in each plot and all understory algae within quadrats were removed by hand and/or a dive knife and placed into mesh bags (mesh size approximately 500 μm). We did not sample encrusting coralline algae. The samples were taken back to the lab and washed in fresh water to remove sediments and other organic materials, such as gastropods. The algae were identified then dried at 70˚C for 48 hours (Wright and Davis 2006). Dry weights were taken according to the functional groups (Steneck and Dethier 1994). We weighed *Caulerpa* rhizomes and *Sonderopelta coriacea/Peyssonnela novaehollandie* separately as the function of these species may be different to other understory algae.

Survivorship of microscopic Ecklonia sporophytes

Culturing

Microscopic sporophytes were out-planted in June and November 2014. For these experiments, sori were collected from 12 adult thalli at Fortescue Bay at approximately 10 m depth. They were kept in a cool dark container filled with seawater and transported back to the laboratory where they were kept overnight with aeration. Each sorus was cut to approximately 50 x100 mm in size, cleaned in sterilised 0.2 µm filtered seawater with 1% of Betadine® solution and rinsed with fresh filtered seawater. The sori were then placed between pieces of absorbent paper and left in a dark cool (12 ˚C in June/17 ˚C in November) room overnight. The next morning, sori were placed into pre-sterilised f/2 seawater media (herein SWM, Andersen 2005) under a constant light intensity of approximately 133 µmol s^{-1} m^{-2} for one hour to release zoospores. Sori were discarded after an hour and zoospore density measured by using a hemocytometer. A stock solution of approximately 7,000 zoospore ml^{-1} was created (by adding filtered seawater) to maximise germination success (Tatsumi and Wright 2016). 50 ml of sterilised f/2 SWM was added to prepared culture jars that had a frosted microscope slide (25 x 75 mm) on the bottom. A few jars also had a 10 mm^2 coverslip which could be removed and examined to monitor development during the culturing. Jars were placed under a light intensity of ~133 µmol photon m^{-2} sec^{-1} on a 12 : 12 light : dark cycle. The temperature was set to ambient seawater temperature at the time of the experiment (12 ˚C for June and 17 ˚C for November). Both trials were maintained in the laboratory for 6 weeks before out-planting of microscope slides supporting sporophytes. Examination of test coverslips after 40 days verified the development of micro-sporophytes.

Transportation and out-planting

The day before out-planting, we removed microscope slides from the culture jars and secured them onto PVC panels (380 x 115 x 5 mm) with elastic bands ready for out-planting. Five microscope slides were attached to each panel along with three 'control' slides which had been kept in the f/2 SWM for the duration of culturing, but with no zoospores added. The prepared panels were kept in filtered seawater in large transport containers overnight and transported to Fortescue Bay the next day. In the field, the panels were removed from the containers and taken underwater immediately. Three panels each containing the eight slides were installed in each plot onto pre-installed mesh racks. The racks were weighed down with heavy chain and positioned haphazardly in the plots. The panels were installed 30 cm above the substratum to avoid grazing by the sea urchin, *Centrostephanus rodgersii*. We observed a number of gastropods (mostly *Clanculus limbatus* but also *Bellastraea aurea* and *Phasianotrochus* spp.) on the panels during the out-planting, but we did not attempt to exclude them. The microscope slides were left on the rack for 42 days, collected and transported back to the laboratory in the same transport containers filled with seawater. In the laboratory, the number of sporophytes on each microscope slide were counted with the naked eye or under a dissecting microscope when necessary. No *Ecklonia* recruits were observed on any control slide in either trial.

Natural macroscopic recruitment of Ecklonia

All macroscopic *Ecklonia* recruits (stages 1 and early 2) were counted in 0.25 m^2 quadrats placed randomly in each plot on 6 occasions over the two years of the experiment. These counts were made in June, September, November 2014, and in May, September and December 2015.

Survivorship, height, growth and erosion of macroscopic Ecklonia recruits

Four times during the experiment (266 days from September 2014, 203 days from February 2015, 196 days from May 2015 and 118 days from September 2015), 10 stage 1 recruits (as defined by Kirkman 1981, < 150 mm in length) were transplanted into each plot. On each occasion approximately 150 recruits were collected near the experimental plots and at the same depth. The blade length (bottom of the central lamina to tip) of each juvenile was measured and a 3 mm diameter hole was punched into the central lamina 5 mm above the top of the stipe. The method was modified from Larkum (1986) to best suit the juveniles, and allowed growth to be measured without damaging the small thalli. These juveniles were then attached to a 1.5 m long, 6 mm diameter 3-strand polyethylene rope by untwisting strands and placing the holdfast between them before tightening it again. Ten juveniles, 100 mm apart, were attached to each rope. These were kept overnight in a dark cool container filled with seawater. One rope was then installed in each plot secured on a fixed anchor point at one end and with a dive weight at the other end. Tensioning the rope held the juveniles in place and also ensured they remained on the substratum. The survivorship, lamina length (mm), growth rate (mm day^{-1}) and rate of erosion (mm day^{-1}) of these juveniles were measured at each visit (2-3 measurements for each trial) to the site, but only the final measurement of survivorship, lamina length, overall growth, and erosion rate were used in the analyses. Growth rate was measured as the distance from the hole to the bottom of the central lamina minus 5 mm (as the holes were punched 5 mm above the bottom of the central lamina) and calculated as growth (mm/day) for each time period based on the number of days since the previous visit. When the hole was no longer able to be seen (due to erosion of the thallus), we used the thallus height to calculate growth rate (therefore, potentially underestimating growth). Erosion was calculated by

$$Rate\ of\ erosion = \frac{(Length_{t0} + growth_{t1} - Length_{t1})}{Number\ of\ days}$$ as mm per day,

where t_0 is the measurement from previous visit and $t1$ is the new measurement. When the hole was eroded, we used height from the previous measurement minus 5 mm to calculate erosion rate.

Analysis

Differences in sediment deposition and water flow were determined using a two-factor ANOVA (Density [fixed] x Month [random]) while differences in sediment accumulation and irradiance (from the LI-COR measurements) were determined using a two-factor nested ANOVA (Density [fixed] x Month [random] with plots [random] nested within the Density x Month interaction). Sub-canopy light reduction data was arsine transformed. ANOVA assumptions were tested using diagnostic plots, model residuals, and data were transformed as required based on the maximum log-likelihood λ value from Box-Cox plots. Where significant overall effects were evident, differences between means were tested with Tukey's post-hoc test. As Tukey's test is a conservative approach, a less conservative test (least significant difference; LSD) was used when Tukey's could not identify differences between means. We attempted transforming data for scouring, but they were highly skewed and did not meet ANOVA assumptions. Thus, we tested these data with the non-parametric Kruskal-Wallis test followed by Mann-Whitney U tests to compare treatment means (Quinn and Keough 2002, Dytham 2011, Beermann et al. 2013). In the non-parametric tests, we did not include each sensor card as a nested factor as this analysis cannot accommodate nested designs.

Species-area curves showed that an 0.25 m^2 area was not a good representation to capture understory algal species richness associated with each density treatment, therefore, total species richness pooled across five quadrats per plot was used to run a one-factor ANOVA

across densities. Biomass data were analysed by one-factor nested ANOVA (plot [random] nested within the density treatments [fixed]) on total biomass and on the separate biomass of five functional groups (see Steneck and Dethier 1994, Appendix A) per quadrat. The five functional groups were articulated calcareous algae, corticated macrophytes, brown leathery macrophytes and red leathery macrophytes and *Sonderopelta coriacea*/ *Peyssonnelia novaehollandiae*. *S. coriacea* and *P. novaehollandiae* had their own category as their functional morphology is different from other macrophytes. Understory community assemblage (biomass by functional groups) after 24 months of density manipulation was determining using multivariate analyses. Canonical analysis of principal coordinates (CAP) was used to distinguish differences in multivariate community assemblage using Bray-Curtis similarity with no data transformation. A nested permutational analysis of variance (PERMANOVA) was also run on these data, with plot nested within the density treatments.

There was generally low survivorship of microscopic sporophytes to 40 days, and there were many zeros for replicate slides (72% in winter and 65% in summer). Therefore, we used generalised linear models (GLM) to test differences in survivorship of microscopic recruits among density treatments. The winter sample was tested using a Poisson and summer sample tested with quasi-Poisson depending on the degree of data dispersion (Zuur et al. 2009) with a log-link. The density of natural recruits was analysed with two-way nested ANOVA (plot [random] nested within density [fixed] x month [random]).

Because stage 1 *Ecklonia* juveniles were transplanted for different amounts of time, differences in juvenile performances (survivorship, lamina length, growth rate, and erosion rate) were determined at the end of transplantation period separately for each cohort. A single factor ANOVA with the factor adult *Ecklonia* density (fixed) was used to determine

differences in survivorship among plots, while nested ANOVAs (plot [random] nested within density treatment [fixed]) were undertaken to determine differences in height, growth rate, and erosion rate. Because growth and erosion were calculated as rates per day for the duration of transplanting, we also tested for differences among cohorts using a two-factor ANOVA (density [fixed] x cohort [random]). ANOVA assumptions were tested by diagnostic plots, model residuals, data were transformed as required based on the maximum log-likelihood λ value from Box-Cox plots. Because transplanted juvenile *Ecklonia* ranged in size between 50 - 150 mm, we conducted a preliminary analysis which revealed that growth rate had no significant relationship with initial size (lamina length) of the transplanted recruits, except for the September 2014 cohort. However, the initial lamina length of transplanted sporophytes in September 2014 cohort was not significantly different among plots. Tukey's post-hoc tests were performed when significant overall effects were detected. All ANOVA analysis was conducted using R studio (ver. 1.0.136) and R (ver. 1.68) with the *MASS* package and the Canonical analysis of principal coordinates (CAP) and Permutational multivariate analysis of variance (PERMANOVA) routines were performed using PRIMER 6 (ver. 6.1.18) & PERMANOVA+ (ver. 1.0.8).

Results

Sediment deposition

There were significant differences among density treatments ($F_{3,39} = 3.176$, $P = 0.034$) and time of the year ($F_{3,39} = 55.001$, $P < 0.001$) for sub-canopy sediment deposition but no significant interaction between kelp density and time ($F_{12,39} = 0.780$, $P = 0.667$). Tukey's post-hoc test could not identify which density treatments differed, but LSD post-hoc test indicated that the plot with medium kelp density had significantly lower sediment deposition compared to all other density treatments (Fig. 2.1a). Sediment deposition during April, June and July were significantly higher than November (approximately double) and March

(approximately 5 times, Fig. 2.1a). Traps accumulated more sediment below the canopy than above (50 to 100 percent higher depending on the month, Fig. 2.1b). There was no effect of kelp sporophyte density ($F_{3,37} = 2.307$, $P = 0.092$) but there was a significant effect of time ($F_{4,37} = 4.548$, $P = 0.004$) for the percentage difference in sediment deposition below the canopy relative to above the canopy, with June significantly higher than March, July and November (Fig. 2.1b). April was not different from any other months. There was no density x time interaction ($F_{12,37} = 0.893$, $P = 0.562$) in the percentage difference in below- versus above-canopy sediment deposition.

Sediment accumulation

Both sporophyte density and time of the year had significant effects on the depth of accumulated sediment in the turf-sediment matrix (density: $F_{3,52} = 8.965$, $P < 0.001$, month: $F_{6,52} = 6.366$, $P < 0.001$) but there was no significant interaction between density and time ($F_{18,52} = 0.735$, $P = 0.791$). Plots with no kelp had significantly higher sediment accumulation than all other treatments (Fig. 2.1c). The mean depth of accumulated sediment in the low density treatment was over 1.5 mm less than the zero density treatment (Fig.2.1c). Although no significant difference was found between the low, medium and high kelp density treatments (Tukey's comparisons > 0.05), the depth of the turf-sediment matrix decreased with increased adult *Ecklonia* density. The mean depth of the matrix with the low density of kelp was approximately 0.8 mm deeper than in the high density kelp treatment ($P = 0.063$, Fig. 2.1c). There was also significant variation among plots within each treatment ($F_{52,600} = 1.854$, $P < 0.001$).

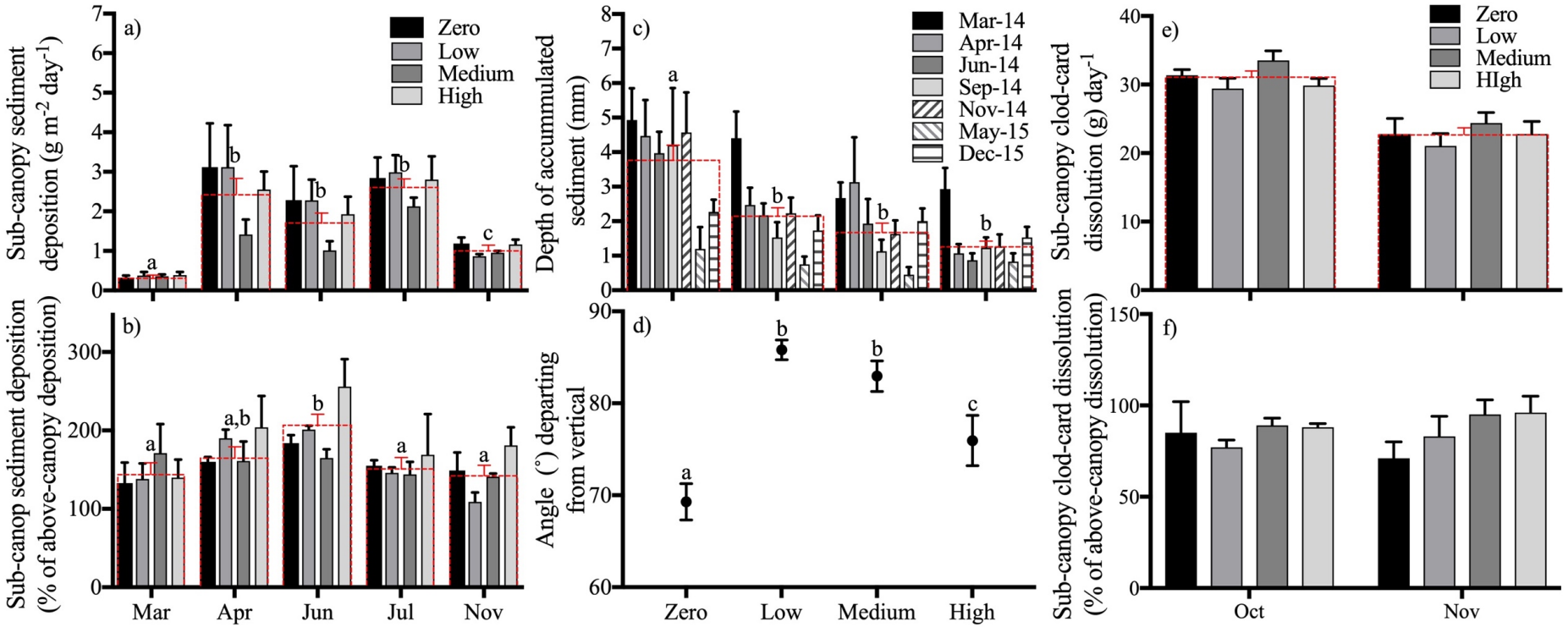

Figure 2.1: The effects of *Ecklonia radiata* density on mean (± SE) sub-canopy (a) sediment deposition rates, (b) relative sediment deposition, (c) sediment accumulation in the turf/sediment matrix, (d) scour, (e) water flow and, (f) relative water flow. The different *Ecklonia* densities were Zero, Low (1-2 thalli m^{-2}), Medium (4-5 thalli m^{-2}), and High (average of 9 thalli m^{-2}). Percentage change of sub-canopy sediment deposition (b) and water flow (f) were compared against paired above-canopy measurements (ambient). That is 100% represents an equal rate of sedimentation or flow and greater or lower amount indicates increased or decreased in the rates respectively'. Scour (d) is indicated by mean angle of wires departing from the vertical position with greater departure from vertical indicating greater scour. Dashed red bars overlaying groups show monthly (a,b,e) or treatment (c) mean. Significant differences detected in Tukey's post-hoc tests are indicated with different letters.

Scour

The amount of canopy scour differed significantly among plots with different densities of adult *Ecklonia* ($x^2_3 = 47.630$, $P < 0.001$). The medium and low density treatments had the highest amount of benthic scour compared to both high and zero kelp treatments (Fig. 2.1d). Not surprisingly, benthic scour was significantly lower in plots with no kelp than in plots with kelp at any densities. Controls (i.e. caged sensors) all showed no deformation (0˚ departure) of the copper wires.

Water flow

There was no significant effect of kelp density on water flow below the canopy ($F_{3,16} = 2.448$, $P = 0.101$), although flow measured from dissolution of clod-card varied significantly with month ($F_{1,16} = 62.418$, $P < 0.001$). Dissolution rate in November was approximately one third less compared to that in October (Fig. 2.1e). The density x month interaction was not significant ($F_{3,16} = 0.408$, $P = 0.749$). Relative water flow below the canopy compared to paired flow above the canopy were not significantly affected by kelp density nor time (interaction: $F_{3,16} = 0.562$, $P = 0.648$, density: $F_{3,16} = 1.605$, $P = 0.228$, month: $F_{1,16} = 0.013$, $P = 0.909$, Fig. 2.1f).

Irradiance

In-situ Odyssey PAR logger showed that from November 2014 to March 2015, plots with no kelp or kelp at low density generally had higher below-canopy PAR (24-hour average) compared to plots with kelp at high density (Fig. 2.2a). PAR in plot with no kelp was usually above 30 µmol m^{-2} sec^{-1} while beneath kelp at natural density, it rarely exceeded 10 µmol m^{-2} sec^{-1}. All treatments other than natural (high) density fluctuated in PAR over time. From

April to September 2015, there were relatively small differences in PAR among the treatments (Fig. 2.2a).

PAR measured using the handheld spherical underwater sensor highlighted a significant density x month interaction for sub-canopy irradiance as well as significant between plot variation (Table 2.1a). In winter, the PAR reached an average of 50 µmol sec^{-1} m^{-2} when there was no *Ecklonia* and decreased by approximately 10 µmol sec^{-1} m^{-2} as density increased (Fig. 2.2b). In spring, the difference between the plots with no kelp and plots supporting *Ecklonia* thalli were much larger (Fig. 2.2d). PAR in plots with no kelp averaged 200 µmol sec^{-1} m^{-2} while below-canopy in plots with kelp at low density was only 60 µmol sec^{-1} m^{-2}. PAR in plots with kelp at medium and high density was lower again (15.8 and 8.9 µmol sec^{-1} m^{-2} respectively) and did not differ significantly from each other. The Tukey's post-hoc test showed that sub-canopy PAR was significantly lower in winter under zero and low density treatments compared to that of spring, while the opposite was observed in plots with kelp at medium density (PAR was higher in winter than spring, Table 2.1b, Fig. 2.2b, d). No difference between winter and late spring were observed under natural density.

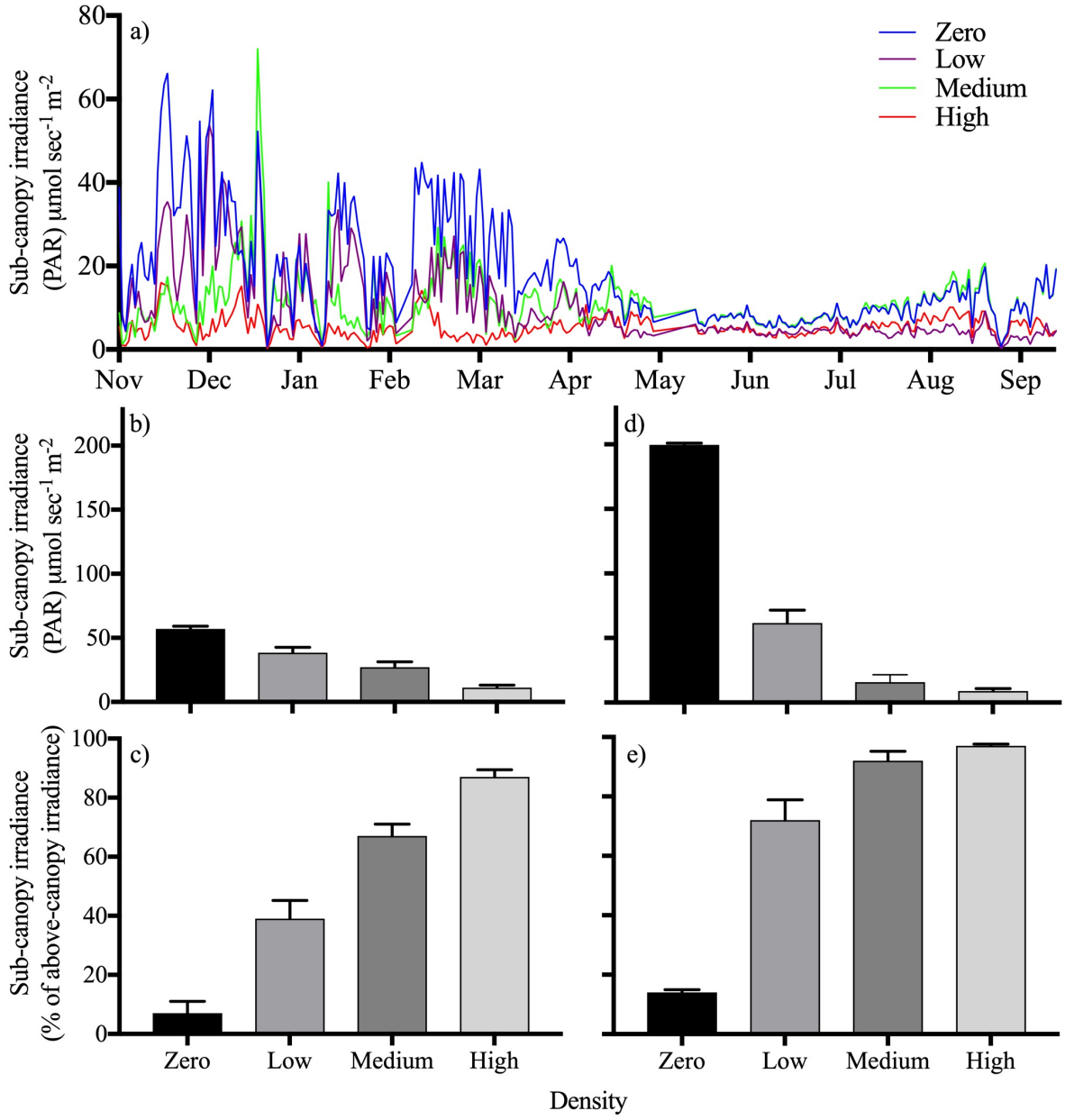

Figure 2.2: The effects of *Ecklonia radiata* thallus density (Zero, Low: 1-2 thalli m^{-2}, Medium: 4-5 thalli m^{-2}, High: average of 9 thalli m^{-2}) on sub-canopy photosynthetic active radiation (PAR). (a) 24-hour daily averages measured by a flat PAR logger from November 2014 to September 2015. Mean ± SE absolute and percentage reduction of sub-canopy PAR as compared to above-canopy PAR were measured on one day in June (b and c) and November (d and e) 2015 using a spherical PAR sensor (~330˚). Significant differences detected in Tukey's post-hoc test are presented in Table 2.1.

Table 2.1: Two-way analyses of variance examining the effects of adult *Ecklonia radiata* density (Zero, Low: 1-2 thalli m^{-2}, Medium: 4-5 thalli m^{-2}, High: average of 9 thalli m^{-2}) and time (June and November 2015) on (a) sub-canopy photosynthetic active radiation (PAR) and b) relative sub-canopy PAR compared to ambient. Abbreviations; Z: Zero, L: Low, M: Medium, H: High.

	Source	df	*F*	*P*
a)	Density	3	3.941	0.145
	Month	1	0.026	0.994
	Den x Mo	**3**	**4.442**	**0.036**
	Plot (Den x Mo)	**14**	**8.604**	**< 0.001**
	Residuals	66		
	Tukey's test:			
	Jun: Z > L = M > N, **Nov:** Z > L > M = N			
	Z: Jun < Nov, **L:** Jun < Nov, **M:** Jun > Nov, **N:** Jun = Nov			
b)	**Density**	**3**	**32.457**	**0.009**
	Month	**1**	**20.669**	**< 0.001**
	Den x Mo	3	0.705	0.565
	Plot (Den x Mo)	**14**	**13.503**	**< 0.001**
	Residuals	66		
	Tukey's test:			
	Month: Jun < Nov			
	Density: (Jun) Z < L < M < N, (Nov) Z < L < M = N			

The percentage reduction in PAR beneath the *Ecklonia* canopy relative to PAR above the canopy was significantly affected by adult sporophyte density and season, but no interactive effect of these factors was found (Table 2.1b). There was approximately 90% reduction in incident light by the natural *Ecklonia* canopy (high density treatment) in both winter and spring (Fig. 2.2c, e). Kelp at medium and low density also reduced light, from 40% reduction in the low density treatment in winter to up to 90% reduction by the medium density canopy in spring. In spring, even in plots with kelp at low density, the canopy reduced PAR by over 70% (Fig. 2.2e). No significant difference was observed in the effect of the medium and natural kelp density treatments on PAR in spring (Table 2.1b). We also observed 7% (winter) and 14% (Spring) light reduction in plots with no kelp which were likely caused by slight differences in the depth (~ 1.5m) between the positions at which the 'above' and 'below' canopy readings were taken.

Understory algae richness, biomass and assemblage

The density of the *Ecklonia* canopy did not affect the average understory algal species richness ($F_{3,8}$=1.880, P = 0.211), but there was significant variation among the plots within each density ($F_{8,47}$ = 3.819, P = 0.002). Zero and low density treatments had 1-2 additional species compared to medium and high density treatments (Fig 2.3a), although these were not statistically significant. In contrast, the biomass of understory algae differed significantly among sporophyte density treatments but there was no significant variation among the plots within density treatments (Table 2.2a). Zero, low and medium density treatments had between 40-50 grams of understory algae per 0.25 m^2 while at natural (high) sporophyte density there was less than 30 grams per 0.25 m^2 (Fig. 2.3b). The difference in understory algae biomass between zero and high sporophyte density treatments was significant, while all other treatment did not differ. There was a significantly higher biomass of brown leathery macrophytes in the zero density treatment compared to all other treatments (Table 2.2e) but no significant density effects were observed for any other groups. Articulated calcareous algae were only common in plots with no kelp, but it was not statistically different among treatments (P = 0.053, see Table 2.2b, Fig. 2.3b). PERMANOVA revealed that overall understory community structure did not differ among different adult *Ecklonia* density treatments at the end of the experiment (after 24 months, *pseudo*-$F_{3,8}$ = 1.312, P = 0.232). Although minor clustering of plots with no kelp can be observed in the CAP result which seems to separate the community assemblage from plots with high kelp density, it is not statistically significant (Fig. 2.3c). There was also no significant variation among plots within treatments (*pseudo*-$F_{8,47}$ = 1.396, P = 0.066).

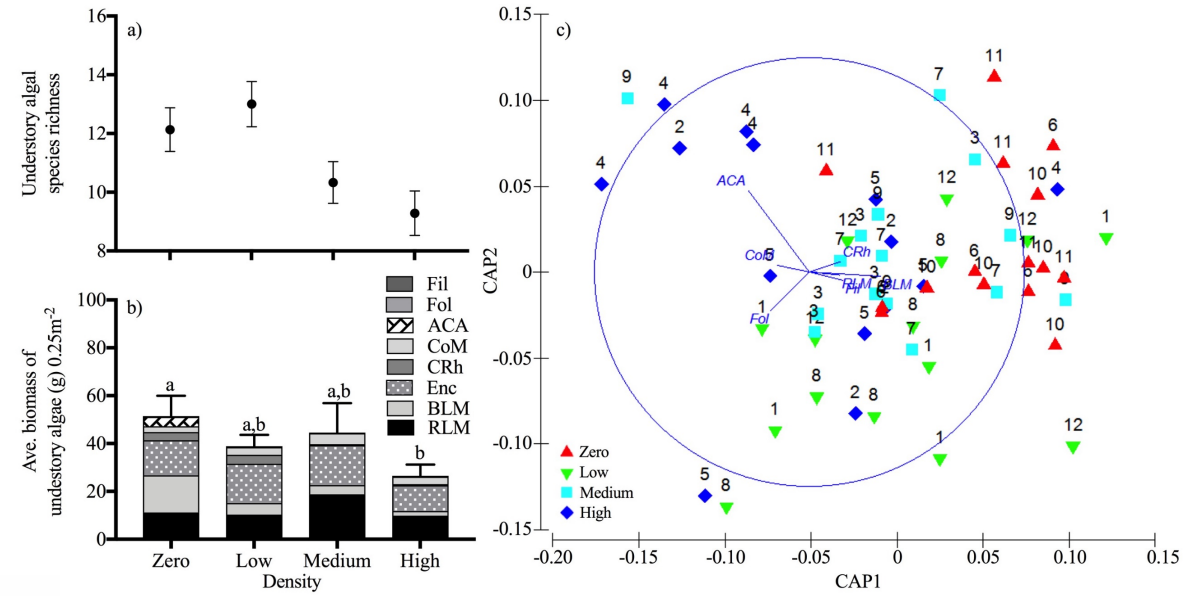

Figure 2.3: The effects of *Ecklonia radiata* thallus density (Zero, Low: 1-2 thalli m^{-2}, Medium: 4-5 thalli m^{-2}, High: average of 9 thalli m^{-2}) on understory algae. (a) mean (± SE) species richness, (b) mean (± SE) biomass, and (c) community structure based on a canonical analysis of principal coordinates (CAP) plot after 24 months of density manipulation. Post-hoc results for biomass (b) show (indicated by different letters) differences among treatments for total biomass. See Appendix A for species in each of the functional groups (Fil: filamentous algae, Fol: foliose and corticated foliose algae, ACA: articulated calcareous algae, CoM: corticated macrophytes, CRh: *Caulerpa* spp rhizome, Enc: *Sonderopelta coriacea* and *Peyssonnelia novaehollandiae*, BLM: brown leathery macrophytes, RLM: red leathery macrophytes). Each point in the canonical analysis of principal coordinates of assemblages (biomass data) represents a quadrat within a plot (numbered) within the density treatments.

Survivorship of microscopic sporophytes

In winter, 3.6 ±1.2 (mean ± SE) sporophytes survived per slide in the plot with kelp at natural (high) density while no sporophytes survived in plots from which kelp was completely removed and less than 0.2 sporophytes survived per slide in the 'medium' and 'low' kelp density treatments (Fig 2.4a). There were also significant differences in survivorship of microscopic sporophyte among plots within kelp density treatments and among racks within plots (Table 2.3). In spring, much higher survivorship was observed in all plots regardless of kelp density, but there was large variation among racks within plots and among plots within

kelp density treatment (Table 2.3). The 'zero' and 'low' kelp density treatments had 22.4 ±7.8 and 18.9 ± 6.5 sporophytes surviving per slide respectively, while under the canopy in plots with kelp at natural density, survivorship was half these numbers. In plots with kelp at medium density, there were only 1.1 ±0.5 surviving per slide. Due to significant variation within racks and plots, we could not detect which means were different from one another.

Table 2.2: One-away analyses of variance examining the effects of adult *Ecklonia radiata* density (Zero, Low: 1-2 thalli m^{-2}, Medium: 4-5 thalli m^{-2}, High: average of 9 thalli m^{-2}) on the biomass of: (a) total understory algae, (b) articulated calcareous algae, (c) corticated macrophytes, (d) *Sonderopelta coriacea* and *Peyssonnelia novaehollandiae*, (e) brown leathery macrophytes and, (f) red leathery macrophytes.

	Source	df	*F*	*P*
a)	**Density**	**3**	**3.282**	**0.029**
	Plot(Density)	8	1.396	0.223
	Residuals	47		
	Tukey's: see Fig. 3b			
b)	Density	3	3.008	0.053
	Plot(Density)	8	2.095	0.084
	Residuals	21		
c)	Density	3	0.076	0.973
	Plot(Density)	8	0.728	0.666
	Residuals	46		
d)	Density	3	0.308	0.819
	Plot(Density)	8	1.099	0.388
	Residuals	35		
e)	**Density**	**3**	**10.371**	**< 0.001**
	Plot(Density)	8	1.916	0.084
	Residuals	40		
	Tukey's: Zero > Low = Medium = High			
f)	Density	3	0.127	0.944
	Plot(Density)	8	0.528	0.829
	Residuals	46		

Natural recruitment

Natural recruitment varied significantly with season ($F_{5,43} = 15.107$, $P < 0.001$), but was not significantly affected by the density of adult sporophyte ($F_{3,15} = 1.737$, $P = 0.202$), and there was no time x density interaction ($F_{15,43} = 1.837$, $P = 0.321$). Post-hoc test revealed that recruitment in June and September 2014 was significantly higher than all other months in

2015 while recruitment in November 2014 was also significantly higher (Fig. 2.4c). Although June 2014 had almost double the recruit density of November 2014 (Fig. 2.4c), post-hoc test did not detect a significant difference between them (Tukey's comparisons, $P = 0.058$). In June and September 2014, there were approximately 15 recruits m^{-1} while recruitment was approximately half that level in November 2014 and less than one-third in May, September and December 2015 (Fig. 2.4c). The density of juvenile kelp (recruits) in plot with natural (high) densities of adult sporophytes did not change in the 12 months after the manipulation, while in plots where adult sporophyte density was manipulated (zero, low and medium density treatments) there was a post-manipulation recruitment spike but then a decline in recruitment over time (Fig. 2.4c). There was a significant variation in recruitment among plots within treatments ($F_{43,268} = 1.554$, $P = 0.020$).

Table 2.3: Analyses of deviance examining the effect of adult *Ecklonia radiata* density on the survivorship of outplanted microscopic sporophytes in June (winter) and November (spring) 2014 for 42 days.

Source	df	Deviance	Residual df	Residual deviance	*P*
Winter sample (Poisson fit)					
Null			168	819	
Density	**3**	**343.81**	**165**	**475.19**	**<0.001**
Plot(Density)	**9**	**211.1**	**156**	**264.09**	**<0.001**
Rack((Plot)Density)	**21**	**182.51**	**135**	**81.54**	**<0.001**
Spring sample (Quasi-poisson)					
Null			206	11356.1	
Density	**3**	**1422.6**	**203**	**9933.5**	**<0.001**
Plot(Density)	**8**	**3953.2**	**195**	**5920.3**	**<0.001**
Rack((Plot)Density)	**23**	**3217.6**	**172**	**2762.7**	**<0.001**

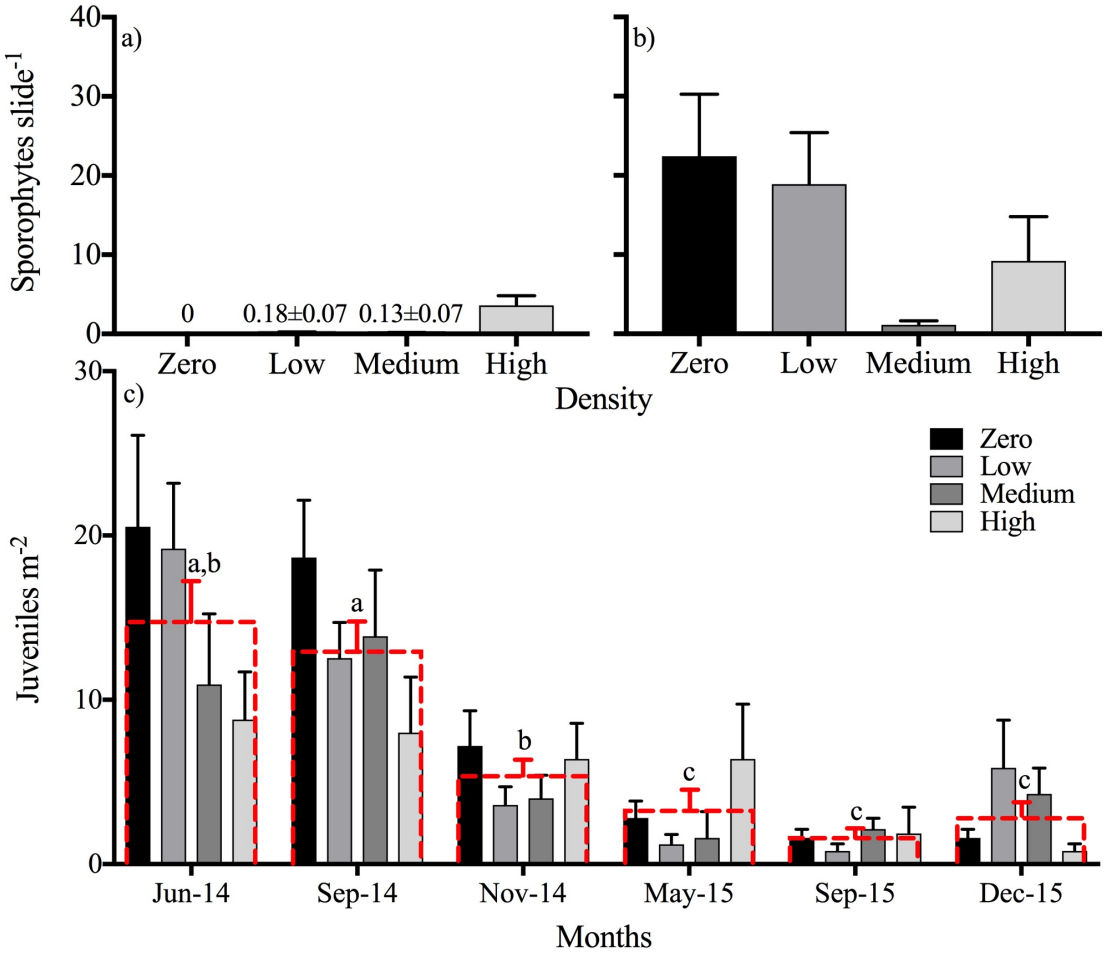

Figure 2.4: Mean (± SE) number of outplanted microscopic *Ecklonia radiata* sporophytes per microscope slide (25 x 75 mm) after 42 days in four adult *Ecklonia* thallus density treatments (Zero, Low: 1-2 thalli m^{-2}, Medium: 4-5 thalli m^{-2}, High: average of 9 thalli m^{-2}) in (a) June 2014 and (b) November 2014. (c) The number (mean ± SE) of macroscopic juvenile *E. radiata* in plots with different densities of adult sporophytes surveyed at six times between June 2014 and December 2015. Dashed red bars overlaying groups show monthly means across treatments. Significant differences among months are indicated with different letters.

Survivorship, height, growth and erosion of macroscopic recruits

There were no significant differences in the survivorship of transplanted recruits among different treatments of canopy density for all cohorts (Table 2.4). Survivorship of juveniles transplanted in September 2014 was high and similar across treatments to 83 days (~80%) and declined to ~ 50% after 266 days (Fig. 2.5). The other cohorts were transplanted for less time and showed similar overall patterns. Juveniles transplanted in both February and May

2015 survived at levels of ~ 50-70% to 203 and 196 days respectively while those transplanted in September 2015 showed similar survivorship to other cohorts at the same duration.

The average lamina length of juveniles in the plot with kelp at natural (high) density after 266 days was significantly smaller than in all other treatments for the September 2014 cohort (< 200 mm compared to ~ 300 mm, Fig. 2.5, Table 2.4). A similar trend was observed for the September 2015 cohort, although the effect was not significant (average height < 150 mm compared to > 200 mm, although $P = 0.059$, Fig. 2.5, Table 2.4). The February 2015 and May 2015 cohorts showed no effects of adult kelp density on lamina length. There was significant variation among plots within treatments for all cohorts except February 2015 (Table 2.4).

Overall, the growth rate of transplanted juveniles was affected by both adult kelp density and the date on which they were transplanted (different cohorts), but there was no significant 'kelp density x cohort' interaction (Table 2.4). The growth rate of the juveniles in the plots with adult sporophytes at natural (high) density was significantly lower (1.127 ± 0.075 mm day^{-1}) than that in plots with adult kelp at zero (1.61 ± 0.055 mm day^{-1}), low (1.565 ± 0.069 mm day^{-1}) and medium (1.458 ± 0.093 mm day^{-1}) density, which did not differ. Across the different cohorts, the September 2014 cohort had the highest growth rate of 1.771 ± 0.078 mm day^{-1} followed by May 2015 (1.514 ± 0.052 mm day^{-1}) and September 2015 (1.427 ± 0.065 mm day^{-1}). No statistical difference was observed between May 2015 and September 2015 cohorts. The February 2015 cohort had significantly slower growth than all other cohorts (1.061 ± 0.084 mm day^{-1}). There was also significant variation among plots within treatments of adult kelp density (Table 2.4).

The overall rate of erosion was significantly affected by the date on which juveniles were transplanted (cohort), but not by adult sporophyte density (Table 2.4). There was also significant variation among plots within density treatment, but there was no interaction between adult kelp density and cohort. The September 2014 cohort had significantly higher erosion rates (1.161 ± 0.052 mm) compared to all other cohorts (February 2015: 0.652 ± 0.04, May 2015: 0.765 ± 0.057, September: 0.809 ± 0.141 mm day^{-1}), and no differences were found among these cohorts. Nested one-way ANOVAs on individual cohorts revealed no effects of adult density on erosion rate for all cohorts except February 2015 cohort where erosion rates in plot with adult sporophyte at zero and low density were significantly higher than in plots with adult kelp at medium and high density (Fig. 2.5, Table 2.4).

Table 2.4: Analyses of variance examining the effects of adult *Ecklonia radiata* sporophyte density (Zero, Low: 1-2 thalli m^{-2}, Medium: 4-5 thalli m^{-2}, High: average of 9 thalli m^{-2}), and plot-nested-within-density, on the survivorship, lamina length, growth rate and erosion rate of stage 1 juvenile *E. radiata* transplanted in (a) September 2014, (b) February 2015, (c) May 2015, and (d) September 2015. For growth and erosion rates, two-factor ANOVAs (i.e. density x cohort) were also performed (e). Abbreviations; Z: Zero, L: Low, M: Medium, H: High; S14: September 2014, F15: February 2015, M15: May 2015, S15: September 15 cohort.

	Survivorship			Lamina length			Growth rate			Erosion rate		
Source	**df**	***F***	***P***	**df**	***F***	***P***	**df**	***F***	***P***	**df**	***F***	***P***
a) Density	3	0.014	0.998	3	2.873	**0.025**	3	1.747	0.244	3	2.508	0.143
Plot (Density)	-	-	-	**8**	**2.605**	**0.021**	**7**	**8.094**	**<0.001**	**7**	**3.111**	**0.010**
Residuals	8			41			40			41		
Tukey:				Z = L = M > H								
b) Density	3	2.579	0.126	3	1.122	0.350	3	1.630	0.295	**3**	**11.193**	**0.012**
Plot (Density)	-	-	-	8	1.372	0.234	5	2.252	0.074	5	0.815	0.549
Residuals	8			46			31			30		
Tukey:										Z = L < M = H		
c) Density	3	1.277	0.346	3	0.897	0.484	3	0.646	0.613	3	0.099	0.958
Plot (Density)	-	-	-	**8**	**2.922**	**0.009**	6	1.363	0.252	**6**	**3.787**	**0.004**
Residuals	8			53			42			41		
Tukey:												
d) Density	3	0.891	0.486	3	3.789	0.059	3	0.868	0.496	3	1.080	0.411
Plot (Density)	-	-	-	**8**	**2.718**	**0.012**	**8**	**9.887**	**<0.001**	8	1.688	0.125
Residuals	8			66			63			49		
Tukey:												
e) Density	-	-	-	-	-	-	**3**	**7.006**	**0.010**	3	0.018	0.997
Cohort	-	-	-	-	-	-	**3**	**5.361**	**0.005**	**3**	**7.302**	**0.001**
Density*Cohort	-	-	-	-	-	-	9	0.461	0.887	9	1.339	0.266
Plot(Density*Cohort)	-	-	-	-	-	-	**26**	**4.820**	**<0.001**	**26**	**2.043**	**0.004**
Residuals	-			-			176			161		
Tukey:							Density: Z = L = M > H			Cohort: S14 > F15 = S15 = F15		
							Cohort: S14 > M15 = S15 > F15					

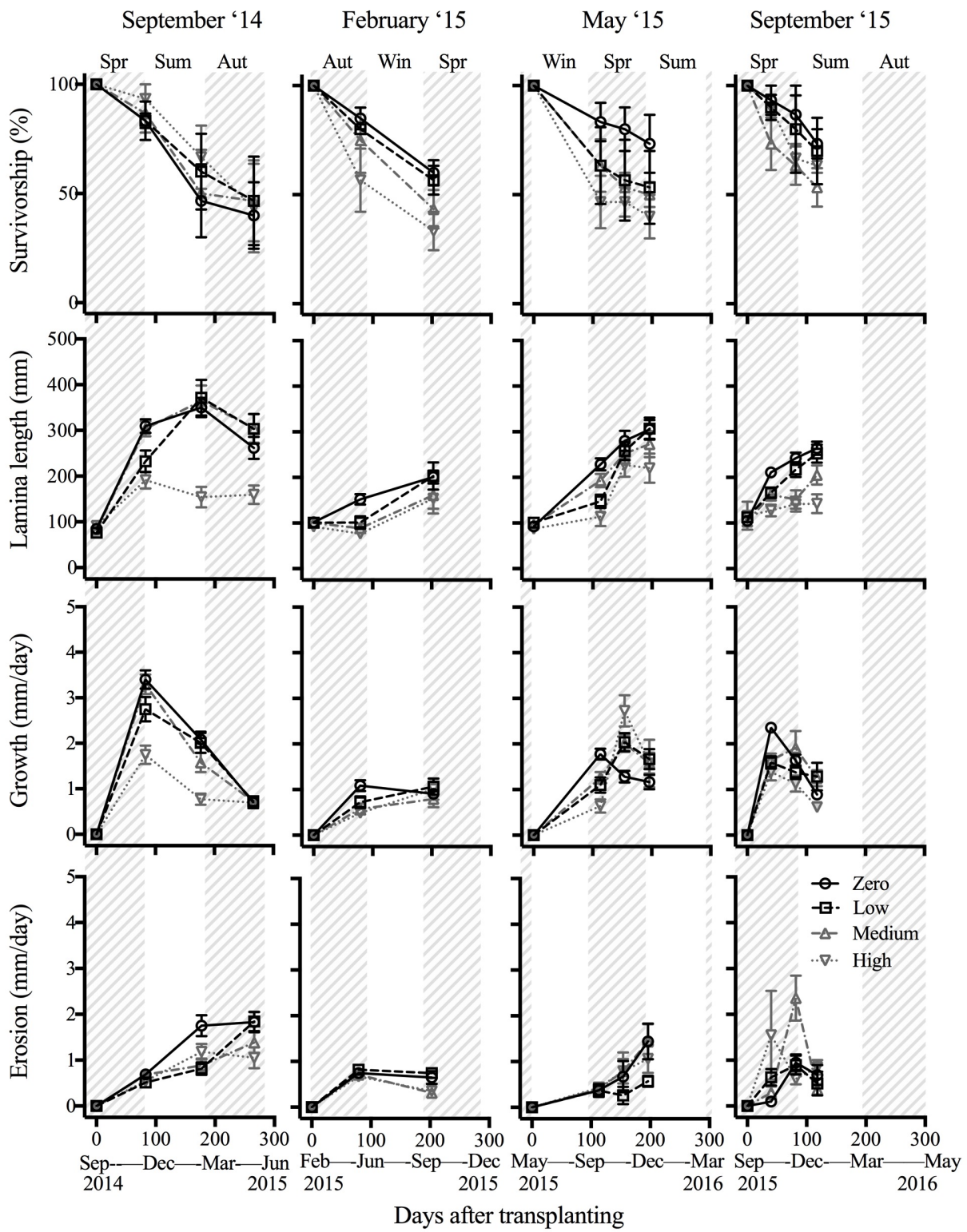

Figure 2.5: Mean ± SE survivorship, lamina length, growth rate, and erosion rate of stage 1 juvenile *Ecklonia radiata* transplanted into plots with different adult *Ecklonia* thallus density (Zero, Low: 1-2 thalli m^{-2}, Medium: 4-5 thalli m^{-2}, High: average of 9 thalli m^{-2}). See Table 2.4 for results of statistical analyses.

Discussion

Our study has identified density-dependent engineering of light and scouring levels on the benthos, depth of accumulated sediment (decreased with increased kelp density) but no density-dependent effects on sediment deposition and water flow. The complexity of these responses coupled with the strong temporal and small-scale (metres to tens-of-metres) variation in most of the variables measured appears to have resulted in limited effects on the understory community, with understory algal biomass only increasing significantly in the absence of *Ecklonia*. Similarly, the demographic response of *Ecklonia* recruits was not strongly dependent on the density of adult sporophytes but was often variable at small spatial scales highlighting the importance of processes acting at the microscale in affecting individual recruits.

Ecosystem engineering of abiotic factors

Sub-canopy PAR was the abiotic factor that responded most consistently to a reduced density of adult *Ecklonia*. Both the *in-situ* flat PAR and spherical PAR sensors indicated pronounced reductions in PAR below the canopy with increasing kelp density, although the relative reduction was lower in June than in November, suggesting seasonal variation in *Ecklonia*'s capacity to reduce sub-canopy PAR. Even though ambient light is much lower in winter, kelp canopy cover is often highest in spring/summer and lowest in winter (Kirkman 1984, Reed and Foster 1984, Dayton et al. 1992, Fairhead and Cheshire 2004) due to storms (Kennelly 1987) and seasonal patterns in sporophyte growth. At our site, the reduction in PAR beneath the canopy relative to above-canopy levels was greater when measured in November (late spring), which coincided with the time of the year when growth is highest, than in June (winter). For example, in the low-density treatment with only 1-2 thali m^{-2}, the canopy reduced PAR by 70% in November compared to only 40% in June. Importantly, in

plots with sporophytes at natural (high) density, PAR reduction beneath the canopy was similar across time regardless of ambient light, providing consistently low sub-canopy light condition across all seasons that rarely exceeded a daily average of 10 μmol m^{-1} s^{-1}. Higher growth of juveniles was observed in warmer months across all kelp density treatments (Fig. 2.5) while absolute PAR can be lower during the warmer months at medium and high kelp densities which indicate that light may not be the only factor contributing to higher growth rate. Sub-canopy light reduction is commonly observed in other kelp species, for example, *Macrocystis pyrifera* significantly reduces ambient PAR creating self-shading and limiting understory algal settlement (Gerard 1984, Graham et al. 2007).

Sedimentation often strongly affects benthic community assemblages (Eckman et al. 1989, Irving and Connell 2002, Wernberg et al. 2005, Irving et al. 2009). From April 2014, despite sediment deposition being similar in all treatments other than the medium density which had lower sediment deposition, accumulation was significantly higher in the plots with no adult kelp. This indicates that sediment deposition rates are not a good indicator of sediment accumulation, and that there are other factors contributing to sediment accumulation. High accumulated sediment is often observed on turf algal dominated reefs as turf algae trap sediments and create a turf sediment matrix (Airoldi 2003, Valentine and Johnson 2003, Birrell et al. 2005, Strain et al. 2015). High levels of accumulated sediment has also been observed after the loss of the *Ecklonia* canopy (Wernberg et al. 2016) and at a reduced *Ecklonia* density (Wernberg et al. 2005). The loss of the canopy will remove the effect of benthic sweeping of sediment by laminae and increase light which enhances the growth of filamentous turf algae (Kennelly 1989, Valentine and Johnson 2003). Previous studies have found higher sediment deposition related to reduced flow under canopy forming kelp

(Eckman et al. 1989, Madsen et al. 2001). Given flow was not reduced by density it is unsurprising that we did not find an effect on sediment deposition.

Benthic scour from sweeping by laminae was also affected by adult sporophyte density being lowest at high density compared to medium and low kelp densities. High-density *Ecklonia* forests may have lower scour because neighbouring thalli block each other's movement and capacity to reach the benthos, reducing the contact of the abrasive lamina or laterals with the substratum. As density decreases, there may be less baffling of lamina movement and thus more scour. The finding of a decrease in scour with high sporophyte density contradicts the prediction of Connell (2003) of higher scour from physical abrasion with higher *Ecklonia* density. In addition to the density of adult sporophytes, a range of factors are likely to influence the amount of scour beneath the canopy including thallus morphology, wave exposure and topography (Seymour et al. 1989, Toohey et al. 2004, Wernberg et al. 2005, Toohey et al. 2007). *Ecklonia* has large morphological variation that is influenced by latitude, longitude, wave exposure, depth, density and whether they occur in monospecific or mixed algal stands (Fowler-Walker et al. 2005a, b, Fowler-Walker et al. 2006, Mabin et al. 2013). *Ecklonia* at our study site (43˚S) have a relatively long stipe length of over 300 mm compared to *Ecklonia* in mainland Australia (33-34˚S) which typically has stipe lengths less 100 mm (Fowler-Walker et al. 2005b). Although *Ecklonia* with short stipes and with the canopy laying nearer the benthos may have higher scour than *Ecklonia* with longer stipes (Kennelly 1989), our results indicate significant amounts of benthic abrasion for Tasmanian *Ecklonia*. Moreover, our experiment also revealed that some physical abrasion occurred in the absence of *Ecklonia* and an understory community (which was experimentally removed). Deformation of wires occurred in plots without kelp was likely to have been caused by drift

algae which are abundant in kelp forests, moving about in surge conditions (Harrold and Reed 1985).

We did not observe a significant change in water flow under different kelp densities, but the dissolution rates of clod cards indicated strong temporal variation occurred. Dissolution rates below the canopy were approximately 90% of the rates above the canopy (across different treatments and times). As plots were established within a large continuous healthy *Ecklonia* forest, an adult sporophyte density effect on water flow may not have been revealed due to the size of our plots (5 m x 5m) being too small. Although there are some studies showing macrophytes reducing water flow (Madsen et al. 2001, Layton et al. in review), different densities of *Ecklonia* on natural reefs have previously been shown not to influence relative water flow which instead, was more affected by local topographical formations (Wernberg et al. 2005, Toohey and Kendrick 2008). Nonetheless, a study by Layton et al. (in review) on artificial reefs with *Ecklonia* at different densities and patch sizes removed the influence of reef topography on flow and identified that sub-canopy water flow is reduced by both kelp density and patch size. Water motion plays an important role in macroalgal production. Lower water velocity reduces zoospore dispersal, increasing concentration (Cie and Edwards 2011) and reduces resuspension of sediment particles, decreasing turbidity and increasing light intensity (Dring and Lüning 1994). Macrophytes also have increased photosynthesis and nutrient uptake in slower flow as a reduced diffusion boundary layer increases flux of solutes (Hurd 2000, Madsen et al. 2001). It is not surprising that there was no effect of kelp density on sediment deposition as flow rates were all similar across all density treatments, but interestingly, scour rate was significantly affected by sporophyte density which supports the idea that in dense stands, scour is reduced because adjacent sporophytes inhibit each other's movement.

Effects on understory algae community

Understory algal community structure did not differ under different densities of adult *Ecklonia* despite that previous studies have identified changes in the understory with declining density of *Ecklonia* (Toohey et al. 2004, Flukes et al. 2014). However, we did identify a significantly higher biomass of both total understory algae and brown leathery macrophytes (in particular *Sonderopelta coriacea/Peyssonnelia novaehollandiae, Zonaria* spp and *Halopteris paniculata*) in the absence of kelp. Similarly, the removal of canopies of *Macrocystis pyrifera* and *Pterygophora californica* in California resulted in an increase in the density of understory brown algae (Clark et al. 2004). A high cover of brown leathery macrophytes can form a new canopy layer and reduce benthic light to almost zero (Tatsumi and Wright 2016). This may impact other understory algae, in particular, red algae which often respond slowly to kelp canopy clearance (Clark et al. 2004). The loss of canopy can also affect sessile invertebrates, such as sponges and bryozoans, which may decline as the community shifts towards a foliose algal dominated condition (Flukes et al. 2014).

Although light is the primary factor determining the abundance and composition of the understory algae assemblage, high light may result in additive effects on understory algae if the increase in understory algae also increases the capture of sediments to enhance the turf-sediment matrix. Canopy gaps increase the cover of filamentous and foliose algae which form the turf sediment matrix (Airoldi 1998, Valentine and Johnson 2004, Irving and Connell 2006), and sediment accumulation can prevent development of kelp recruits (Valentine and Johnson 2004, Watanabe et al. 2016). For example, the settlement of *M. pyrifera* is reduced by 70% by a sediment layer and can be further reduced with increased sediment loads (Geange et al. 2014).

Effects on recruitment and post-recruitment performance of juvenile E. radiata

Survivorship of outplanted microscopic juveniles was very low with only 0.1% of juveniles surviving in the winter cohort (no data available for spring cohort) after 6 weeks of outplanting. Low survivorship of microscopic algal recruits is commonly observed (Schiel and Foster 2006) and has previously been measured as 0.6% survivorship to 35 days for *Ecklonia* (Tatsumi and Wright 2016). This high mortality is likely influenced by multiple abiotic factors including scour, light, water flow and sedimentation as well as grazing and intra- and interspecific competition (Vadas Sr et al. 1992). There was limited evidence that the survivorship of outplanted microscopic sporophytes was related to density-dependent changes in these factors and the microscale variability in the survivorship of these early life-cycle stages highlights the importance of small-scale refuges for microscopic kelp recruits. It is also important to note that although rates of microscopic juvenile survivorship is low, juvenile densities m^{-2} are relatively high, that is the lowest number of surviving sporophytes (0.18 ± 0.07 per slide) we observed in plots with low kelp density in June still equates to 96 juvenile sporophytes m^{-2}.

Scour causes high mortality of early-post settlement recruits (Chapter 3). We identified less scour at natural (high) adult sporophyte density compared to medium and low adult kelp densities, but at both times the survivorship of microscopic recruits outplanted into the different density treatments did not correlate with that pattern. In June 2014, the highest survivorship occurred in the natural density plots, but this did not happen in November 2014 when overall survivorship was greater. Thus, a high adult density may provide a benefit at certain times only. Moreover, given that a high adult kelp density also results in more adult holdfasts, the high density of kelp recruits often observed around adult holdfasts may reflect a local refuge from scour and/or benthic grazing (Anderson et al. 1997). In addition, the consistent low light conditions under the natural canopy (i.e. the high density treatment,10

μmol m^{-1} s^{-1}) may have inhibited the development of sporophytes, consistent with observation that higher light levels (> 40 μmol m^{-1} s^{-1}) are required for germination and growth of microscopic sporophytes (Tatsumi and Wright 2016). In contrast, gametophyte density is enhanced by low light. We did not measure accumulated sediment on the slides, but it is unlikely that sedimentation contributed greatly to the high mortality of post settlement recruits. There are strong negative impacts of sedimentation on pre-settlement kelp zoospores (prevention of attachment) but weaker effects on post-settlement gametophytes (Geange et al. 2014).

In addition to abiotic factors, biotic factors such as grazing and competition may have also been important. Grazing by sea urchins can transform Tasmanian kelp forests into urchin barrens (Ling et al. 2009, Johnson et al. 2011) but smaller grazers can also have significant impacts on microscopic kelp recruits. For example, the gastropod *Tegula atra,* grazes both gametophytes and micro- and macroscopic sporophytes of *Macrocystis pyrifera*, significantly reducing their survivorship (Henriquez et al. 2011, VanMeter and Edwards 2013). We observed grazers such as *Clanculus limbatus* and *Bellastraea aurea* on our experimental racks and they may have grazed some of the outplanted microscopic sporophytes.

Natural recruitment did not differ among adult density treatments but was influenced by large between plot variations suggesting that suitable microhabitats may also be important for recruitment. There were also large seasonal differences in recruitment. The initial high recruitment in June and September 2014, particularly in the plot with no kelp and low adult density plots, may have reflected a response to increased light following our removal of adult sporophytes. The plots at zero and low adult sporophyte density had more than double the recruit density as the unmanipulated plots in June. High kelp recruitment following canopy

removal is commonly observed (Dayton et al. 1984, Johnson and Mann 1988, Taylor and Schiel 2005, O'Connor and Anderson 2010, Flukes et al. 2014). Microscopic stages of kelp can have a dark-adapted resting stage and gametophytes can survive and germinate after a year of dormancy once adequate light becomes available (Novaczek 1984, Edwards 2000). The high density of macroscopic recruits observed initially following canopy removal likely reflected the rapid growth of existing recruits. Interestingly, the density of recruits decreased over time, particularly in the kelp removal treatments, suggesting a self-thinning mechanism which has also been reported for other kelp in similar circumstances following canopy removal (Johnson and Mann 1988). With sufficient light, it takes approximately 4 months for microscopic *Ecklonia* juveniles to grow to macroscopic stage 2 juveniles (Kirkman 1981) indicating that a sub-canopy "seed bank" can quickly restore the canopy. However, continuous canopy removal may result in a loss of the seed bank over time, limiting recruitment even when there is sufficient light.

Dean et al. (1989) found that survivorship of macroscopic juvenile *Macrocystis pyrifera* was negatively affected by canopy thickness and grazing. However, in the present study, the absence of kelp canopy did not affect *Ecklonia* survivorship of recruits, even when transplanted in summer, suggesting that *Ecklonia* recruits were resilient to high light in zero- and low-kelp density treatments. We generally measured > 50% survivorship of these recruits to 200 days across all cohorts, which compares with similarly high survivorship of *Ecklonia* stage 1 recruits reported previously (Wood 1987, Toohey and Kendrick 2007). However, the overall lamina length and growth (September 2014 cohort only) of juveniles was negatively influenced by density, with significantly slower growth of juveniles under high (natural) adult densities compared to all other treatments despite lower benthic light levels with higher densities of adult sporophytes. Low light levels likely caused slower growth of recruits in

high (natural) density plots in September 2014 cohort. Greater growth rate and lamina length observed in the September 2014 cohort may also reflect higher light saturation points compared to other cohorts as September 2014 cohort was the only cohort transplanted for an entire summer and the amount of light needed for light saturation increases with the temperature (Staehr and Wernberg 2009). Bearham et al. (2013) found negative effects of high water temperature on *Ecklonia* growth, which does not match with our finding, but their upper temperature range was much higher (> 21˚C) than we observed in summer and may have exceeded a physical optimum for *Ecklonia*. Finally, erosion rates in the February cohort were lower in plots with zero and low adult sporophyte density suggesting a possible effect of adult kelp density on erosion of juveniles during autumn and winter. Observed higher erosion rates in the September 2014 cohort (the only cohort that experienced a full summer) was in line with the finding of Miller et al. (2011) who argue that higher erosion is observed during warmer months, decreasing biomass of individual thallus during summer and in winter, it minimises drag forces of winter storms, making them less susceptible (de Bettignies et al. 2013).

Conclusion

We have shown that *Ecklonia* modifies PAR and scour at the benthos in a density-dependent manner, and that sediment accumulation also decreases with increased density of adult sporophytes. However, sediment deposition and water flow were not modified by the density of adult *Ecklonia* but there was a clear seasonal signal in both of these factors. Strong temporal and small-scale spatial variability limited identification of possible engineering effects of *Ecklonia* on associated understory species, but overall biomass was greater when *Ecklonia* was absent than when there was a closed canopy. Small-scale spatial variability also made it hard to detect the demographic response of *Ecklonia* recruits to different canopy

densities, but also emphasised importance of microhabitats within a kelp forest. We conclude that some of the important abiotic parameters are engineered in a density-dependent manner and the capacity of *Ecklonia* to modify certain abiotic factors (sub-canopy light) changes with season. Different life stages of *Ecklonia* (microscopic recruits to fully grown adults) can be enhanced or inhibited from engineering feedbacks, but the overall resilience of the kelp forest at a population level is increased via engineering of the local environment by adult sporophytes (e.g. quick recovery of canopy after canopy removal). A reduction in *Ecklonia* density will reduce its capacity to engineer the local environment in this way, and therefore could reduce its resilience and stability. Maintenance of healthy kelp populations is critical to maintain the positive feedbacks that act to maintain *Ecklonia* forests in the face of competition from other algae such as turfing species.

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Appendix

Appendix A: List of macroalgae species identified in understory algae assemblage under different density of *Ecklonia radiata* in Fortescue Bay, Tasmania, Australia.

Phaeophyta	***Rhodophyta (continued)***	***Rhodophyta (continued)***
Corticated macrophytes	Foliose/Corticated foliose	Leathery macrophytes (continued)
Halopteris paniculata	*Halymenia ssp.*	*Callophyllis rangiferia*
Zonaria turneriana/angustata	*Schizoseris hymenema?*	*Delisea pulchra*
	1 x unidentified sp.	*Dictyomenia harveyana?*
Leathery macrophytes		*Euptilota articulata*
Acrocarpia paniculata	Corticated macrophytes	*Gloiocladia polycarpa?*
Carpoglossum confluens	*Camontagnea oxyclada?*	*Hypnea ramentacea?*
Carpomitra costata	*Champia viridis*	*Phacelocarpus sp. 1*
Cystophora sp.	*Lenormandia sp. 1*	*Phacelocarpus sp. 2*
Sargassum sp.	*Lenormandia sp. 2*	*Placomium angustum*
Sargassum vestitum?	*Mastophoropsis canaliculata*	*Plocamium dilataum*
2 x unidentified spp.	*5 x Rhodymenia spp.*	*Plocamium leptophyllum*
	8 x unidenfitied spp.	*Ptilonia australasica*
Chlorophyta		*Rhodophyllis multipartita*
Filamentous	Articulated calcareous algae	*Sarcothalia crassifolia*
Chaetomorpha billardierii	*Haliptilon roseum*	*Thamnoclonium dichotomum*
	Corallina officinalis?	*4 x unidentified spp.*
Foliose/Corticated foliose	*Cheilosporum sagittatum?*	
Ulva spp.	1 x unidentified sp.	
Rhodophyta	Leathery macrophytes	
Encrusting	Areschougia congesta?	
Sonderopelta coriacea	*Ballia callitricha*	
Peyssonnelia novaehollandiae	*Callophyllis lambertii*	

Chapter 3. Interactive effects of canopy-driven changes in light, scour and water flow on self-recruitment in kelp

Masayuki Tatsumi, Cayne Layton, Matthew J. Cameron, Victor Shelamoff, Craig R. Johnson and Jeffrey T. Wright

Abstract

The marine ecosystem engineering kelp, *Ecklonia radiata*, forms habitat and influences associated communities by altering resource availability and modifying abiotic conditions. Although the importance of facilitation by ecosystem engineers on associated species is well recognised, mechanisms underpinning intra-specific feedbacks on the engineer's own demographic rates (recruitment, growth and survivorship) are not well known. Here, we tested the interactive effects of three abiotic factors engineered by *E. radiata* on the survivorship and growth of outplanted microscopic sporophytes of *E. radiata* in two experiments: 1) crossed treatments of light (ambient vs. low) and scour (present vs. absent) under ambient flow, and 2) crossed treatments of light (ambient vs. low) and flow (ambient vs. low) in the absence of scour. The abiotic factors were manipulated on experimental racks and the survivorship and growth of sporophytes measured after three and six weeks of being outplanted. These abiotic factors engineered by *E. radiata* acted in isolation or interactively to influence the survivorship and growth of early post-microscopic sporophytes. In experiment 1, after only three weeks, scour decreased survivorship of sporelings by > 50% and by 6 weeks the best sporeling survivorship occurred in the absence of scour under low light/ambient flow – similar to the abiotic conditions under an intact canopy. In experiment 2, the treatments only affected sporeling survivorship at 6 weeks and indicated higher survivorship under ambient flow/low light and low flow/ambient light conditions. In contrast to survivorship, growth of sporophytes was almost entirely related to light with sporelings under ambient light approximately 50% larger than those under low light after three weeks and up to four times larger after six weeks. Although the different aged sporelings varied slightly in their response to the manipulated abiotic conditions, reduced scour (for survivorship) and ambient light (for growth) appear crucial for maximising *Ecklonia* recruitment. Thus a healthy adult forest can provide a microenvironment that enhances survivorship of these microscopic sporelings, suggesting a positive feedback, but an increase in light, such as when a gap opens in the canopy, is required for high growth.

Introduction

Ecosystem engineers form physical habitat and influence associated communities by altering resource availability or modifying abiotic conditions (Jones et al. 1994). The structural complexity provided by autogenic engineers often enhances species diversity and these facilitative effects can be far-reaching with entire communities dependent on the presence of the engineer (Silliman et al. 2011, Byers et al. 2012). Although there are a number of mechanisms by which autogenic engineers facilitate associated species, the two main ones are via a reduction in abiotic stress and the provision of a refuge from predation (Crooks 2002, Bruno et al. 2003, Altieri et al. 2010, Wright et al. 2014).

Although the importance of facilitative effects of ecosystem engineers on associated species is well recognised (Jones et al. 1994, Coleman and Williams 2002, Gilad et al. 2004, Berke 2010, Petrosillo and Zurlini 2016), intra-specific facilitation via environment-engineering feedbacks (Jones et al. 2010) has received less attention despite it likely to be crucial for the stability and resilience of habitat-forming ecosystem engineers. Environment-engineer feedbacks occur when the ecosystem engineer species changes the biotic and abiotic environment and these changes affect its own recruitment, survivorship and reproduction (Jones et al. 2010). The mechanisms underpinning environment-engineer feedbacks are not well understood, although examples include where oyster reefs provide shelter for conspecific larvae in shell crevices increasing their recruitment success (O'Beirn et al. 2000), and where seagrass beds reduce water velocity and trap sediment and organic matter which decreases mortality from erosion (De Boer 2007, Folmer et al. 2012). Feedbacks mechanisms are likely to be specific to each engineering species, therefore identifying those mechanisms for ecosystem engineers will be important in understanding how they contribute to their resilience and stability.

Kelps (Order Laminariales) are dominant marine ecosystem engineers on temperate shallow subtidal reefs and provide primary production and structure that support a diverse assemblage of associated species (Steneck et al. 2002, Graham et al. 2007, Steneck and Johnson 2014). Healthy kelp forests create a closed canopy which modifies a range of abiotic factors including light, water flow, sediment deposition/accumulation and scour (Eckman et al. 1989, Kennelly 1989, Wernberg et al. 2005), and changes in these parameters can all potentially feedback to influence kelp recruitment. Of these factors, light appears to be the most predictable and least variable, with healthy canopies typically blocking up to 90 percent of the ambient light (Gerard 1984, Reed and Foster 1984, Kennelly 1989, Wernberg et al. 2005) which may benefit the microscopic haploid stage which in many kelps are susceptible to high light levels (Novaczek 1984, Altamirano et al. 2004). In addition, the reduction in light reduces the abundance of turf and other understory algae which compete with kelp recruits (Kendrick et al. 1999, Clark et al. 2004, Toohey et al. 2004, Flukes et al. 2014, Benes and Carpenter 2015, Tatsumi and Wright 2016). Reduced water flow under canopies has also been documented (Layton et al. in review), but this appears variable and less predictable (Wernberg et al. 2005, Toohey and Kendrick 2008). Similarly, a high kelp canopy cover can reduce the amount of sediment present on the benthos and accumulating within the turfing algal matrix (Wernberg et al. 2005) but is also highly variable in time (Layton et al. in review, Chapter 2). Finally, scour from kelp can strongly influence understory communities (Kennelly 1989, Connell 2003) and is lower with a full canopy cover compared to a reduced cover (Chapter 2).

Ecklonia radiata (herein referred as *Ecklonia*) is the most wide-spread ecosystem engineering marine alga in southern Australia, dominating much of the Great Southern Reef (Womersley 1967, Bennett et al. 2016). *Ecklonia* provides an important habitat for many understory algae,

vertebrates and invertebrates including commercially important species (Johnson et al. 2005). *Ecklonia* is an autogenic engineer and its canopy (structure) modifies key abiotic variables such as light, scour, water flow, and sedimentation (Kennelly 1989, Kendrick et al. 1999, Toohey et al. 2004, Layton et al. in review) which might result in positive direct (reduced scour, sedimentation and water flow at high density) and indirect (e.g. reduced light restricting understory algal growth and thus, competition; Tatsumi and Wright 2016) effects on its own recruits. However, with the exception of low light (Tatsumi and Wright 2016), how those changes might feedback to affect the recruits of *Ecklonia* is unknown and moreover, the interactive effects have not previously been studied.

Ecklonia in Australia currently faces a number of threats including ocean warming, over grazing by range-expanding sea urchins, and more severe and frequent disturbances (Ling and Johnson 2012, Smale and Wernberg 2013, Vergés et al. 2016, Wernberg et al. 2016, Provost et al. 2017). These threats can all result in a reduction in the canopy cover of *Ecklonia* and thus a change in the abiotic environment beneath the canopy. This study examined the interactive effects of three of the abiotic changes engineered by *Ecklonia* on its own recruits. Specifically, we manipulated scour, light and water flow in a field experiment and determined their effects on the early post-recruitment survivorship and growth of outplanted microscopic *Ecklonia* sporophytes. We determined: 1) the interactive effects of changes to light (ambient vs. low) and scour (present vs. absent) under ambient flow, and 2) the interactive effects of changes to light (ambient vs. low) and flow (ambient vs. low) without scour. We did not assess the 3-way interaction between scour, light and water flow as scour will be a function of water flow (i.e. high water flow would always result in high scour), so we first examined the effects of light x scour and then of light x water flow independent of scour.

Materials and methods

Study species and site

Ecklonia radiata has a typical kelp lifecycle with microscopic male and female gametophytes and micro- and macroscopic sporophyte stages (Kirkman 1981, Novaczek 1984). Reproductive sporophytes release free-swimming zoospores. Once settled, zoospores develop into male or female gametophytes. The male gametophytes produce motile antherozoids that fertilise non-motile eggs (oogonia) on the female gametophyte which then develop into juvenile sporophytes (Womersley 1990). In this study, we examined effects of the engineered abiotic factors on sporophytes in the field after 40 days culture in the lab. The field experiment was conducted in Fortescue Bay, Tasmania, Australia (Latitude: -43.126813, Longtitude: 147.958211) between September to October 2015. The site had a flat sandy bottom at 10 m depth and was approximately 5 m from the boundary of a healthy kelp forest. Thus, we established the experimental treatments without any engineering effects of adult canopy, but under similar ambient abiotic conditions.

Experimental design

Experimental set-up and manipulation of abiotic factors

The experiment consisted of six treatments (1: ambient light, scour present, ambient flow, 2: low light, scour present, ambient flow, 3: ambient light, scour absent, ambient flow, 4: low light, scour absent, ambient flow, 5: ambient light, scour absent, low flow, 6: low light, scour absent, low flow) established on a metal rack (6.4 x 1.6 m) placed on the sandy bottom and elevated by approximately 30 cm from the bottom to avoid excessive sedimentation (Fig. 3.1). The rack was constructed to hold 52 replicate ‘slide holders’ which held microscope slides on which sporophytes were growing (only 48, 1: n = 8, 2: n = 8, 3: n = 12, 4: n= 12, 5: n = 4, 6: n = 4, were used in the experiment). Each slide holder was a 210 x 290 mm long

PVC panel that contained 12 frosted microscope slides (25 mm x 75 mm, in two rows of six, 10 slides with cultured sporophytes plus two control slides with no sporophytes). The microscope slides were attached to the slide holder with large elastic bands (two elastic bands for each row of six microscope slides) with the elastic bands covering 5 mm of each end of the slides. Above each slide holder requiring manipulation of the abiotic environment, a frame (310 mm long x 230 mm wide x 80 mm high) constructed from 15 mm diameter PVC was attached to the rack over the slide holder to enable the various manipulations of the abiotic environment required for the different treatments. The PVC frames were secured with cable ties to the rack and treatments positioned randomly. The frame was not attached to slide holders which required no manipulation of abiotic factors.

Light

To manipulate light levels, two layers of fibreglass insect screen were attached on the top of the frames (Fig. 3.1A). Light intensity beneath the mesh was measured for the duration of the experiment (a reading taken every 15-minutes of accumulated light) by installing odyssey loggers on the rack with or without a frame for manipulation of abiotic factors but did not have slide holder in place. Beneath the mesh there was a 24-hour average daily light intensity of 9.9 ± 0.26 μmol photon m^{-2} sec^{-1} which is a similar light level to that which occurs beneath a full *Ecklonia* canopy in Fortescue Bay at the same time of the year (Chapter 2). The mean 24-hour average daily light intensity of ambient light treatment (28.30 ± 0.69 μmol photon m^{-2} sec^{-1}) was significantly higher than the low light treatment with mesh (t = 8.541, df 52.204, $p < 0.001$). The average maximum daily light intensity was also significantly higher under ambient light treatment (127.40 ±6.96 μmol photon m^{-2} sec^{-1}) than low light treatment (47.34 ±3.51 μmol photon m^{-2} sec^{-1}, t = 10.268, df 57.641, $p < 0.001$). To ensure

sediment did not accumulate on the mesh during the experiment we brushed it away by hand frequently (every 7-14 days).

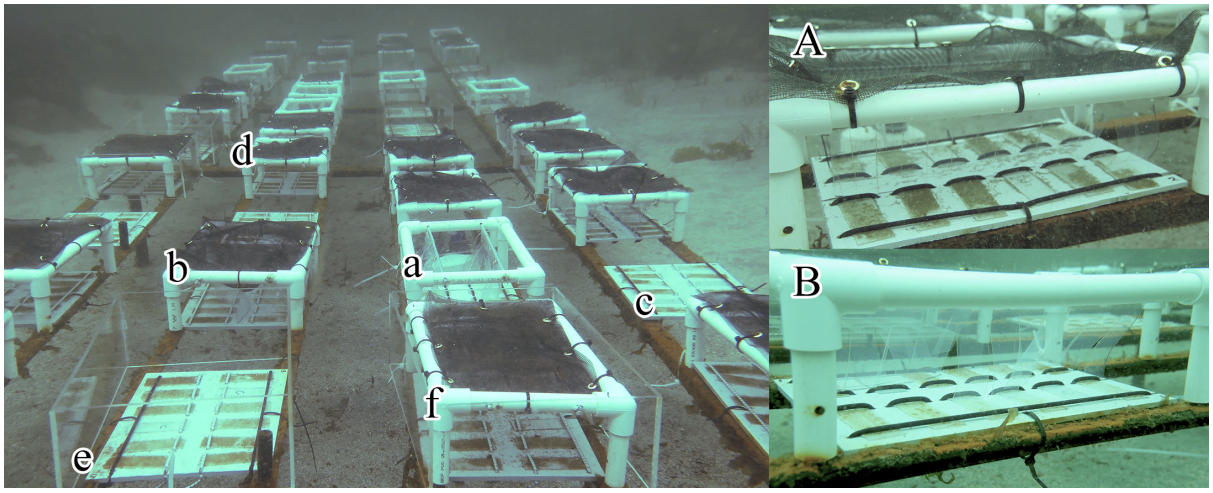

Figure 3.1: A rack for outplanting of microscopic sporophytes showing replicate slide holders and frames to manipulate abiotic factors attached over replicate slide holders. a) Scour present, ambient light, ambient flow, b) scour present, low light, ambient flow, c) scour absent, ambient light, ambient flow, d) scour absent, low light, ambient flow, e) scour absent, ambient light, low flow and f) scour absent, low light, low flow. A) shows shading cloth providing low light condition and B) shows a close-up photo of straps mimicking scour.

Scour

Scouring was mimicked by hanging a series of transparent plastic strips (80 mm long x 10 mm wide, 0.2 mm thick) above microscope slides (Fig. 3.1B). Plastic strips were loosely attached to an elastic cord that stretched the length of the frame so that they moved in the water flow and scoured the surface of the slides. There was one row of plastic scour strips positioned directly above each row of microscope slides. To test the effectiveness of the scour treatment, small panels (white PVC panels cut to the size of microscope slides) painted with non-water based black ink were placed under the different treatments (six slides each on four slide holders for both scour treatments) plus a control (six slides each on two slide holders with mesh cages to protect them from any scour) and left for 3 days. The percentage loss of the paint in the central 1 x 1 cm area on each panel was analysed by using ImageJ 1.50 (the same version was used for all other ImageJ analyses in this paper), and data were arcsine

transformed to meet assumptions of ANOVA. Although plastic strips seemed to produce weaker scouring force than the *Ecklonia* canopy, treatment effects were significantly different ($F_{2,57}$ = 335.736, $P < 0.001$: scour present > scour absent > control: Tukey's post-hoc test) with 98.38 ±0.67 % loss of ink in the 'scour present' treatment, 10.67 ±2.33 % in the 'scour absent' treatment and 5.16 ± 5.16 % loss in the 'control'. The loss of some ink cover on the 'scour absent' treatment appeared to be caused by scouring from sediment and drift algae, and the loss of ink from the control appeared to be from sediment scour (personal observations).

Water flow

Water flow was manipulated by installing 120 mm high x 5 mm thick clear Perspex panels around the perimeter of replicates (Fig. 3.1e). The top of the cage was left open. The effectiveness of this treatment was tested using plaster blocks (clod-cards), approximately 125 cm^3 in size. These blocks were made by pouring a mixture of plaster ($CaSO_4$):water at a ratio of 3:1 into square moulds with a bolt installed in each block before solidifying. These were dried at room temperature for 24 hours, the mould removed and then dried at 50 °C for another 24 hours. Any excess plaster was removed to standardise the shape and the blocks were then weighed and attached in the centre of the slide holder. In the field, the plaster blocks were placed within ambient or flow-restricted treatments (n = 6 of each) prior to the outplanting of sporophytes and left for 3 days. They were collected, dried at 50 °C for 24 hours and weight loss determined. Dissolution of plaster was higher for ambient flow (64 ± 0.5% loss of initial weight) than in the treatment where flow was restricted (60 ± 1% loss, t_{10} = 2.27, $P < 0.046$) indicating that the Perspex baffles significantly reduced water flow even though the differences between treatments were quite small.

Sedimentation

We initially planned to test effects of sedimentation as a factor influencing growth and survivorship. However, since we could not provide an effective or realistic way to manipulate sedimentation in the field, we estimated its effect as a covariate. Sediment accumulation for each block of replicates was measured on one of the control slides (without sporophytes) at 3 and 6 weeks into the experiment. These 'control' slides were collected individually and carefully placed into an individual container for transportation back to the lab. The central 1 x 1 cm area of the slides was assessed first for any natural recruits of *Ecklonia* (no sporophytes were found on any of the control slides) then the sediment on entire slide was scraped into a pre-weighted aluminium tray and dried at 70 ˚C for 48 hours. The dry weights of the sediment from each slide were compared among treatments at weeks 3 and 6. These data were not included in further analyses as there was no difference in sedimentation rates among any treatments at either time (week 3: $F_{5,42} = 0.444$, $P = 0.815$, week 6: $F_{5,39} = 0.953$, P = 0.458).

Culturing

Reproductive tissue from 12 adult *Ecklonia* was collected at Fortescue Bay in July 2015. The tissue was transported back to the lab in seawater in a dark container and kept overnight at 12 ºC with aeration. Sori were cut down to approximately 5 x 10 cm in size, cleaned in 0.2 µm filtered seawater with 1% Betadine® and rinsed in fresh 0.2 µm filtered sea water. Clean sori were placed in between clean paper towels and kept in a 12 ºC dark room overnight to enhance zoospore release. All semi-dried sori were then soaked in two litters of pre-sterilised f/2 seawater media (SWM) and kept under constant light (approx.133 µmol photon m^{-2} sec^{-1}) for an hour to release zoozpores. Sori tissue was then removed from the zoospore solution and zoospore density determined using a haemocytometer. Culturing was done in UV

sterilised 500 ml jars that had a frosted microscope slide placed on the bottom and filled with the f/2 SWM. A coverslip was also placed in 30 jars (haphazardly selected) to monitor the growth of microscopic *Ecklonia* and to assess the timing for the outplanting. Zoospore solution was added to each jar to make the concentration of zoospore approximately 7,000 ml^{-1} to maximise the germination success (Tatsumi and Wright 2016). Lids were placed on jars to reduce contamination and evaporation of the SWM. Jars were then placed under the constant light (approx.133 µmol photon m^{-2} sec^{-1}) on a 12:12 light:dark cycle. Jars were monitored every ~ 7 days and after 40 days five photos of ten randomly selected coverslips were taken under 40x magnification to assess whether there were sufficient sporophytes available for outplanting. There were 4.16 ± 0.52 mm^{-2} sporophytes on slides prior to outplanting.

Outplanting

Every replicate consisted of a PVC slide holder to support 10 slides with sporophytes plus 2 control slides (no sporophytes). Slides with sporophytes and control slides were haphazardly selected and randomly positioned on the slide holders in the laboratory. Once all 12 slides were positioned on slide holders, they were placed in filtered seawater overnight and during transit to the field site before being outplanted the next day. The slide holders were taken underwater and randomly positioned and secured on the pre-submerged rack. If subject to any of the six treatments in which abiotic conditions were manipulated, a frame (randomly selected) was then installed over each slide holder to create the desired abiotic conditions. Half of the slides (five sporophytes slides plus a control) were collected three weeks (21 days) after outplanting and the other half collected after a further three weeks (42 days).

Density and size assessment

At 21 and 42 days the slides were collected from each replicate slide holder and placed in a container which allowed transport back to the lab without any loss of sporophytes. The control slides were placed in a separate container to measure natural recruitment (there was no recruitment to any of the control slides) and accumulated sediment. Back in the lab, the number of sporophytes in the central 10 mm x 10 mm quadrat of each slide was counted by the naked eye. Ten individuals were then haphazardly selected from each replicate (not each microscope slide) to determine size by taking photos under a microscope with Canon EOS600D. The surface area of those images was measured using ImageJ.

Statistical analyses

Sporophyte survivorship and size at each time were analysed using nested Analyses of Variance. Because it was not possible to conduct a full 3-way design (see above), we conducted separate ANOVAs to determine the interactive effects of scour x light, and water flow x light (all fixed factors), with replicate slide holders nested within the interactions. The assumptions of ANOVA were tested using diagnostic plots and the data were transformed based on the λ value at maximum log-likelihood from Box-Cox plots as necessary. Tukey's post hoc tests were conducted where we found any significant overall effects. All analyses were conducted using R Studio ver. 1.0.136 with R ver. 3.3.3 and *MASS* package. The figures were produced by GraphPad PRISM 7.0.

Results

Effect of scour x light on sporophyte survivorship (ambient water flow)

After three weeks post-outplanting and under ambient water flow, only scour had a significant effect on survivorship ($F_{1,36} = 23.027$, $P < 0.001$). There was no evidence of any

effect of reduced light ($F_{1,36}$ = 2.630, P = 0.114), no scour x light interaction ($F_{1,36}$ = 35.809, P = 0.220), and differences among slide holders within replicates of scour x light treatments were not significant (nested effect, $F_{36,160}$ = 1.394, P = 0.086). Slides not exposed to scour had 19.1 ±1.7 (mean ± SE) sporophytes per cm^2, more than double the density of sporophytes in the presence of scour (7.9 ±1.6 cm^{-2}, Fig. 3.2a). However, by week 6, there was a significant scour x light interaction ($F_{1,36}$ = 11.253, P = 0.002) as well as a significant nested effect ($F_{36,157}$= 3.29, P < 0.001). The highest survivorship was observed under 'scour absent/low light' conditions which supported 12.9 ±1.5 sporophytes per cm^{-2} (Fig. 3.2c). The three other treatments had significantly lower survivorship, supporting less than half the density of the scour absent/low light treatment (Fig. 3.2c). At six weeks, scour with low light turned out to be the worst combination for survivorship, and this treatment supported significantly fewer sporophytes than 'scour absent with ambient light'.

Effect of flow x light on survivorship (no scour)

There were significant interactions between water flow and light intensity on survivorship of *Ecklonia* sporophytes at the both times (week 3: $F_{1,28}$ = 6.080, P = 0.020, week 6: $F_{1,28}$ = 11.657, P = 0.002), but a significant nested effect was only found in week 6 (week 3: $F_{28,128}$ = 0.847, P = 0.687, week 6: $F_{28,123}$ = 2.198, P = 0.002). In week 3, the mean sporophyte density in ambient flow/low light (21.1 ±2.3 cm^{-2}) and the low flow/ambient light treatment (19.4 ±2.9 cm^{-2}) supported approximately five more juveniles per cm^2 than the other two treatments, but no significant differences were detected in the post-hoc test (Fig. 3.2b). In week 6, there was a similar pattern but the differences among treatments were more pronounced with significantly higher survivorship in the ambient flow/low light and the low flow/ambient light treatments (~ 13 sporophytes per cm^2) compared to the other two treatments (~ 5 sporophytes per cm^{-2}, Fig. 3.2d).

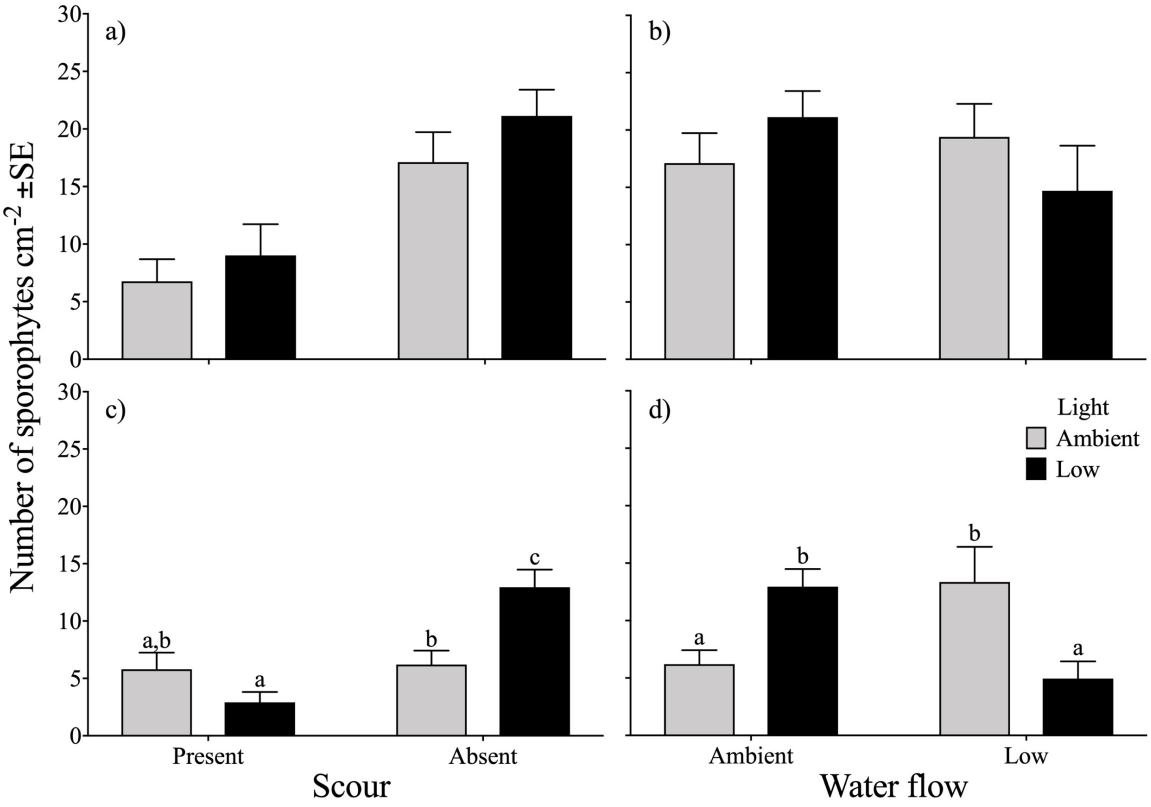

Figure 3.2: Effects of scour, water flow and light on survivorship of outplanted juvenile *Ecklonia radiata* sporophytes after (a, b) 3 weeks, and (c, d) 6 weeks, in the field. We tested the interactive effects of scour (present/absent) x light (ambient/low) and water flow (ambient/low) x light (ambient/low) in separate 2-factor ANOVAs (see methods). Different letters indicate significant difference among the means (Tukey's: $P < 0.05$).

Effects of scour x light on size (ambient water flow)

Scour also had a significant influence on growth by week 3 ($F_{1,31} = 9.230$, $P = 0.005$), with surviving sporophytes approximately 70% larger in the absence of scour (2.726 ±0.154 mm^2) compared to when scour was present (1.589 ±0.107 mm^2). Notably however, there was no effect of scour detectable at week 6 ($F_{1,23} = 0.224$, $P = 0.641$, Fig. 3.3a, c). In contrast, there was a significant effect of light on the growth of sporophytes at both sampling times (week 3: $F_{1,31} = 5.455$, $P = 0.026$, week 6: $F_{1,23} = 67.218$, $P < 0.001$). Under ambient light, 3 weeks into the experiment, sporophytes were approximately 50% larger (2.854 ±0.195 mm^2) than those growing under low light (1.929 ±0.120 mm^2), while by week 6 they were more than three times the size (ambient light: 21.394 ±1.421 mm^2, low light: 6.103 ±0.294 mm^2). No

significant scour x light interactions were observed at either time (week 3: $F_{1,31} = 3.761$, $P = 0.062$, week 6: $F_{1,23} = 3.267$, $P = 0.084$) but there was significant variation among replicates within treatments (week 3: $F_{31,288} = 4.572$, $P < 0.001$, week 6: $F_{23,240} = 7.833$, $P < 0.001$).

Effect of water flow x light on size

There was no effect of water flow on sporophyte size at either time (week 3: $F_{1,28} = 2.01$, $P = 0.167$, week 6: $F_{1,22} = 1.48$, $P = 0.237$). However, sporophytes were approximately 40% larger under ambient light (3.575 ±0.234 mm^2) than low light (2.424 ±0.171mm^2) in week 3 and were almost four times larger under ambient light (24.706 ±1.534 mm^2) than low light (6.103 ±0.293mm^2) in week 6 (week 3: $F_{1,28} = 4.44$, $P = 0.044$, week 6: $F_{1,22} = 75.59$, $P < 0.001$, Fig. 3.3b, d). There were no significant flow x light interactions (week 3: $F_{1,28} = 0.995$, $P = 0.327$, week 6: $F_{1,22} = 0.935$, $P = 0.344$) but there was significant variation among replicates within treatments at both times (week 3: $F_{28,263} = 7.894$, $P < 0.001$, week 6: $F_{22,230} = 7.447$, $P < 0.001$).

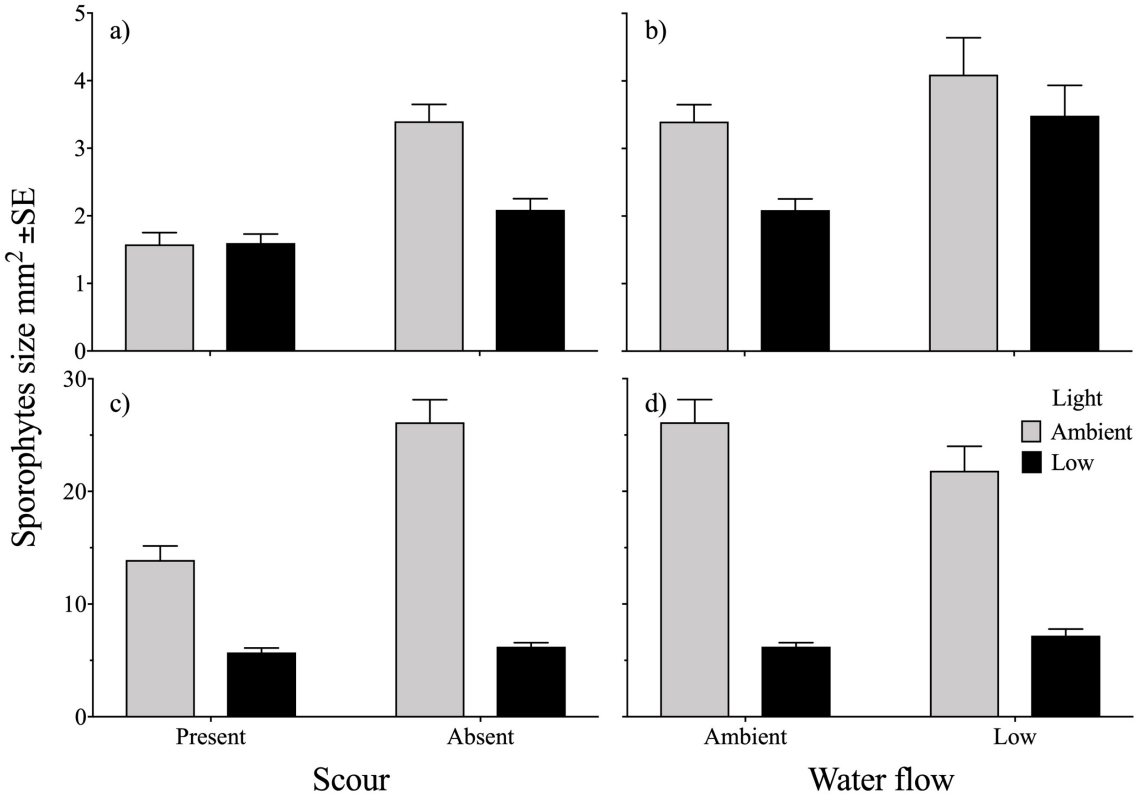

Figure 3.3: Effects of scour, water flow and light on size of outplanted juvenile *Ecklonia radiata* sporophytes after (a, b) 3 weeks, and (c, d) 6 weeks, in the field. We tested the interactive effects of scour (present/absent) x light (ambient/low) and water flow (ambient/low) x light (ambient/low) in separate 2-factor ANOVAs (see methods). Note: data for scour absent-ambient water flow is presented in both the left and right hand panels for each time to enable comparison

Discussion

Our research has identified that the kinds of abiotic factors engineered by adult *Ecklonia radiata* can act in isolation or interactively to influence the survivorship and growth of its recruits at the stage of early sporophytes. Scour reduced early (3-week) post recruitment survivorship by ~ 50%, while after six weeks the highest survivorship was evident in the treatment of no scour and low light (similar to the light intensity beneath a full *Ecklonia* canopy; Toohey et al. 2004, Wernberg et al. 2005). Thus, microsites beneath a closed canopy that escape scour appear important for enhancing the survivorship of microscopic sporophyte recruits. In the absence of scour, the effect of water flow and light on survivorship was

complex, with high survivorship under both 'ambient water flow/low light' and 'low water flow/ambient light' conditions. In contrast to survivorship, increased growth occurred in response to high (ambient) light. While we have shown these factors have important effects on early post-recruitment sporophytes, evidence for a positive environment-engineer feedback effect remains inconclusive. Multiple factors influence the survivorship of *Ecklonia* recruits, but these factors vary in space and time and the scale at which they operate appears crucial.

Survivorship

Scour and light intensity are two of the most prominent abiotic factors engineered by adult *Ecklonia* (Toohey et al. 2004, Wernberg et al. 2005). The strongest effects of scour on survivorship, observed at three weeks into the experiment, highlights its crucial role in sweeping these early life-cycle stages from the substratum. A range of factors are likely to influence the amount of scour occurring beneath the canopy including the density of adult sporophytes and their morphology, wave exposure, and topography (Seymour et al. 1989, Toohey et al. 2004, Wernberg et al. 2005, Toohey 2007). Low-density *Ecklonia* forests have higher scour than high density forests (Chapter 2) possibly because at a high density, thalli block the movement of neighbouring thalli which reduces the contact of the abrasive lamina with the substratum. As the density decreases, there may be less baffling of lamina movement, more scour and thus, lower survivorship of the microscopic recruits.

Given that these high-density forests also have low light at the benthos (Wernberg 2005, Toohey and Kendrick 2008, Layton et al. in review), which is adequate for microscopic sporophyte survivorship but not always growth (Novaczek 1984, Miller et al. 2011, Tatsumi and Wright 2016), these low scour, low light conditions may represent a strong positive

environment-engineer feedback on survivorship of microscopic *Ecklonia* recruits. The effect of scour by lamina on microscopic recruits is likely to vary on small-scales. A single thallus will only abrade a certain part of the substratum depending on its size and morphology (stipe, lamina length, size and density) and the extent to which it is blocked by neighbouring thalli may result in scour 'blind-spots' and micro-scale (mm/cm) variation in mortality from scour. Lower scour in a dense *Ecklonia* forest may increase scour blind-spots and possibly the survivorship of microscopic recruits. In addition, a high density of juvenile *Ecklonia maxima* are often found on holdfasts of mature adults (Anderson et al. 1997). Holdfasts may also provide a scour 'blind spot' if the *Ecklonia* lamina do not sweep the holdfast, but they may also be a good surface for zoospore settlement. Thus, as well as reducing scour, a high density of adult thalli may result in a positive environment-engineer feedback by providing a structure (i.e. holdfasts) for settlement.

Scour had no significant effect on survivorship under ambient light after six weeks, which may have been due to higher growth and the development of holdfasts that attached strongly enough to withstand the scour. Greater holdfast development was observed on the larger sporophytes under ambient light. The attachment strength of a kelp holdfast is largely influenced by the holdfast area (Wernberg 2005) which in turn can be influenced by water motion. Juvenile kelp that grow in wave exposed area are more strongly attached than those which grow in calmer conditions (Milligan and DeWreede 2000). Given the outplanted juveniles were initially grown in a controlled laboratory environment with no water motion, they may have had minimal holdfast development at the time of outplanting and thus may have been susceptible to dislodgement via scour.

The amount of scour by *Ecklonia* thalli will be influenced by their stipe length and individuals with shorter stipes tend to cause more scour (Kennelly 1989, Toohey et al. 2004). Stipe length in *Ecklonia* varies on both large and small spatial scales and is generally positively correlated with latitude but also affected by site-specific factors such as wave exposure, depth, temperature and density (Fowler-Walker et al. 2005, Mabin et al. 2013). *Ecklonia* stands comprised of thalli with short stipes results in the canopy lying closer to substratum, which could result in greater scour (Connell 2003, Altamirano et al. 2004, Toohey et al. 2004). In higher latitudes such as Tasmania, thalli tend to have longer stipes with shorter lamina (Fowler-Walker et al. 2005, Mabin et al. 2013). Nonetheless, scouring of substratum by *Ecklonia* still occurs in Tasmania although it is lowest with the highest density of adult *Ecklonia* (Chapter 2).

Young kelp sporophytes, including *Ecklonia*, are susceptible to high light levels, and their resilience to high light increases as they develop (Wood 1987, Hanelt et al. 1997, Altamirano et al. 2004). Exposure to extremely high light (> 1000 µmol photon m^{-2} sec^{-1}) for less than 15 minutes can be fatal for small (less than 2 mm in length) *Ecklonia* sporophytes (Novaczek 1984). The maximum light level recorded under ambient light during our experiment was 235 µmol photon m^{-2} sec^{-1} which suggests light-induced mortality was unlikely. Although low light (< 10-20 µmol photon m^{-2} sec^{-1}) increases early sporophyte survivorship, increased light is required for subsequent growth and development (Novaczek 1984, Tatsumi and Wright 2016). Although it was not the focus of this study, the low light conditions beneath the canopy (~ 10 µmol photon m^{-2} sec^{-1}) are the best environment for gametophyte survivorship (Tatsumi and Wright 2016) and they can stay viable for several months until adequate light required for optimal growth is available (Novaczek 1984). This effectively provides a sub-canopy 'seed bank' (Carney and Edwards 2006) within healthy kelp forests where

microscopic gametophytes and sporophytes remain dormant until a gap in the canopy creates higher light conditions for development and sporophyte growth. The rapid recovery of *Ecklonia* often observed after canopy removal (Kennelly 1987, Flukes et al. 2014) is consistent with this idea.

Another important effect of low light under a health kelp canopy that might impact the survivorship of microscopic recruits is the inhibition of understory algae by low light. Under a closed *Ecklonia* canopy cover (low light), there is typically a sparse assemblage and low biomass of fleshy, foliose, and turfing understory algae with the benthos largely covered by encrusting algae (Toohey et al. 2004, Wernberg et al. 2005, Flukes et al. 2014). In contrast, as the *Ecklonia* canopy decreases, light increases and the abundance of turfing and foliose algae increases. Understory algae negatively affect *Ecklonia* recruits via competition for light and space but can also cause scour and increase sediment accumulation resulting in decreased *Ecklonia* recruitment and survivorship (Kennelly 1989, Wernberg et al. 2005, Tatsumi and Wright 2016). A thick algal understory can block > 90% of light (Clark et al. 2004, Tatsumi and Wright 2016) and given their potential to also scour the benthos, will create a low-light, high-scour environment which was the worst environment for the survivorship of microscopic *Ecklonia* sporophytes at six weeks. In addition, although *Ecklonia* zoospores and gametophytes are adapted to low light and are able to survive under such conditions, they require minimum light intensity of 40 μmol photon $m^{-2}s^{-1}$ for successful germination (Fairhead and Cheshire 2004a, b, Tatsumi and Wright 2016).

Water flow can also be reduced by *Ecklonia*, but it appears highly variable in time and not strongly related to thallus density in natural stands (Wernberg et al. 2005, Toohey 2007). Flow is also likely to be affected by factors such as topographic formations including

boulders, and reef slope, although under 'standardised' benthic topography such as on artificial reefs, the presence of kelp reduces water flow beneath the canopy (Layton et al. in review). Interestingly, our experiment indicated that any effect of flow on the survivorship of *Ecklonia* recruits depended on light intensity with similar survivorship under ambient flow/low light and low flow/ambient light. Although neither of these treatments were subject to artificial scour, the ambient flow treatment may have been exposed to some scour from drift algae, as we observed some loss of paint when the artificial scour treatment was tested, while the low flow treatment was protected by the Perspex panels. The finding of high survivorship under ambient flow/low light also indicates that the ambient light levels at this depth (approximately 10 m) can be stressful and may make sporophytes more vulnerable to other disturbances. Despite the significant statistical difference in flow, the effect experimental manipulation on flow was minimal with only 4% difference between treatments. It is interesting that the low flow/low light treatment had low survivorship, despite also being protected from scour. We are unsure of the reason for this.

Growth

Growth was highest under ambient light regardless of the amount of scour or water flow. This result is not surprising, and many other studies have previously demonstrated that elevated light results in increased growth of microscopic kelp sporophytes (Novaczek 1984, Miller et al. 2011, Tatsumi and Wright 2016). However, to our knowledge this has not previously been tested in the field with additional abiotic factors manipulated at the same time. In our experiment, PAR never exceeded 250 µmol photon m^{-2} sec^{-1} which is much lower than reported fatal PAR for small *Ecklonia* sporophytes of 1000 µmol photon m^{-2} sec^{-1} (Novaczek 1984). High irradiance (500 µmol m^{-2} s^{-1}) also causes severe photoinhibition in young *Ecklonia cava* sporophytes (Altamirano et al. 2004), and can negatively affect the

germination of gametophytes and post-settlement success of *M. pyrifera* sporophytes (Graham 1996). In the laboratory, microscopic *Ecklonia* sporophytes grow well at PAR up to 133 µmol photon m^{-2} sec^{-1} (Wright and Tatsumi 2016). However, *Ecklonia* can occur in shallower water where much higher (and possibly fatal) light levels occur than those used in this experiment. Because the adult *Ecklonia* canopy can reduce light intensity by 90% (Chapter 2), excessive light may be reduced to acceptable levels for the growth of juvenile sporophytes in these environments. The removal of the adult *Ecklonia* canopy increases tissue damage and photoinhibition, and lowers survivorship, in young sporophytes in shallow water (Wood 1987). An environment-engineer feedback may become less important as depth increases, but since shade from the canopy also decreases understory algal abundance (Toohey et al. 2004), positive feedback effects via a decrease in negative biotic interactions may overpower the negative effects of light on slow growth.

Scour also affected the growth of juveniles within 3 weeks of the experiment commencing. This may be due to larger individuals being removed by scour with smaller individuals being less susceptible and therefore surviving. However, there was no evidence that growth was affected by scour by week 6.

Although no effects of water flow on growth were observed in this experiment, increased water flow enhances nutrient uptake of macroalgae (Hurd 2000) and may also be an important factor affecting the growth of *Ecklonia* recruits. Growth of macroalgae is enhanced by higher water motion (Glenn and Doty 1992) although in *Macrocystis pyrifera,* growth is saturated at a flow rate of 2.5 cm s^{-1} (Gerard 1982) which are relatively calm conditions. If the growth of *Ecklonia* is also saturated in such low water velocity, water motion at our study depth (10 m) should have been adequate to break down boundary layers in both treatments,

which may have contributed to why we could not see any effect on growth. This means that variation in water motion may not be particularly important for *Ecklonia* growth under natural conditions on the open coastline where some surge is normal. The exception may be unusually clam conditions in sheltered waters, or in a highly controlled environment such as in laboratory (Bearham et al. 2013). Given the limited evidence for *Ecklonia* modifying water flow and the lack of clear effects in this experiment, it seems a reduction in water flow is unlikely to provide any feedback effects on growth of juveniles. However, it is important to note that flow enhances canopy movement which can introduce pulses of light to shaded areas. Extreme flow can also increase sediment transfer and turbidity and decrease light intensity (Madsen et al. 2001), which may negatively influence growth.

Conclusion

Juvenile *Ecklonia* have high mortality rates (Tatsumi and Wright 2016), and to maximise their survivorship, an environment-engineer feedback from the adult population may be important. Adult *Ecklonia* cause structural changes, which modify the abiotic and biotic environment beneath the canopy. Those changes can have positive direct (shelter from scour, lower sediment accumulation and protection from photoinhibition; Wood 1987, Wernberg et al. 2005, Layton et al. in review, Chapter 2) or indirect (via interactions with understory algae; Tatsumi and Wright 2016) effects on their recruits. Canopy thinning will reduce the 'engineering effect' of *Ecklonia* and result in higher light, more understory algae, more scour as well as fewer holdfasts (Anderson et al. 1997, Toohey et al. 2004). In our experiment, the best conditions for the survivorship of microscopic sporophytes were no scour, low light and ambient flow, or no scour, ambient light and low flow. These reflect abiotic conditions associated with a fully developed canopy or no canopy respectively. When considering other effects under a closed canopy, such as low understory algae biomass, lower sediment

accumulation (Tatsumi and Wright 2016, Chapter 2), and density-dependent zoospore production (Chapter 4), then the no scour, low light condition would be the ideal condition for recruitment, which emanates from a healthy canopy. Post-recruitment sporophyte growth was best under high (ambient) light highlighting the importance of canopy gaps for growth of recruits. A high density of sub-canopy recruits beneath a healthy adult canopy will ensure dense sub-population and increase the chance of a rapid recovery of the canopy when there is an increase in light, such as when a gap opens in the canopy.

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Chapter 4. Density-dependent and seasonal variation in reproductive output, sporophyte production and performance in the kelp, *Ecklonia radiata*

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Abstract

The common kelp, *Ecklonia radiata*, is an ecosystem engineer of subtidal temperate rocky reefs in southern Australia. Density-dependent changes in the abiotic environment engineered by *E. radiata* may feedback to affect reproduction and subsequent gametophyte and sporophyte recruitment. In this study we examined: 1) how the reproductive capacity of *E. radiata* in the field (the amount of zoospores released per individual) varied with time and adult *E. radiata* density (high [natural], medium and low density plots) and 2) how the recruitment of microscopic gametophytes and sporophytes was influenced by zoospore density in two seasons. Zoospore production per individual varied hugely over time with an indication of a decline in mid-late summer followed by an increase in mid-autumn. However, zoospores production per individual did not vary greatly in relation to adult density with only one month out ten sampled over a 2-year period showing an effect of density (high = medium > low). The lack of a density effect suggests that the total amount of zoospores produced in a *E. radiata* forest is likely to increase as the number of reproductive adults increases. The density-dependent effects of zoospore density on gametophyte and sporophyte recruitment (based on a $\log_2$ zoospore dilution series) indicated both minimum threshold and optimum zoospore densities for sporophyte production, but these varied in time. Almost no sporophytes developed when zoospore density was < 6.5 mm^{-2} in spring < 0.5 mm^{-2} in winter with optimum zoospore densities of 90 - 355 mm^{-2} in spring and 21 - 261 mm^{-2} in winter resulting in relatively high recruitment of between 4 - 7 sporophytes mm^{-2}. Above zoospore densities of 335 mm^{-2} in spring and 261 mm^{-2} in winter, sporophyte densities declined and eventually reached zero at very high densities. These findings suggest that zoospore concentration in the water column should increase with the density of reproductive adults, and high but not extreme, zoospore densities will result in higher sporophyte recruitment.

Introduction

Habitat-forming species, i.e. 'ecosystem engineers' are critical to the functioning of many ecosystems. They modify resource availability, the abiotic environment and influence associated communities (Jones et al. 1994, Coleman and Williams 2002, Hastings et al. 2007). In marine systems, autogenic ecosystem engineers or foundation species (Dayton 1972) including coral, seagrass, mangroves and seaweed provide structure and productivity that supports diverse food webs and underpins extensive ecosystem services (Othman 1994, Wernberg 2005, De Boer 2007, Wild et al. 2011). However, many marine ecosystem engineers are under threat from a range of anthropogenic factors including climate change, habitat destruction and pollution (Scheffer et al. 2001, Ghedini et al. 2013, Strain et al. 2015, Wernberg et al. 2016). Most research on impacts on ecosystem engineer species has focused on understanding the role of these external factors (Coleman and Williams 2002, Elmqvist et al. 2003, Wernberg et al. 2016), however, there may be internal drivers that also affect the demography (reproduction, survivorship and growth) of these species which influences the resilience and stability of the community as a whole.

Internal drivers influencing a marine ecosystem engineer encompass environment-engineer feedbacks (Gurney and Lawton 1996, Cuddington et al. 2009, Jones et al. 2010). This refers to modification of the abiotic environment by an engineer species to create conditions that affect the engineer's own demographic processes including reproductive output, fertilisation rates, post-recruitment survivorship and growth. Environment-engineer feedbacks may be negative or positive. Examples of positive feedbacks include enhanced recruitment of invertebrates in the vicinity of high densities of conspecifics because of the provision of structure or a reduction in abiotic stress (Bertness et al. 1999, Gribben and Wright 2006). Moreover, as the degree of physical modification is often density dependent (Wernberg et al.

2005, Toohey 2007), positive feedbacks may decrease with density. For example, sub-canopy light in a kelp forest increases as kelp density decreases (Layton et al. in review, Chapter 2) and kelp gametophyte recruitment is lower under high light (Tatsumi and Wright 2016).

Kelps are important sub-tidal ecosystem engineers of temperate rocky reefs worldwide (Steneck et al. 2002, Graham et al. 2007, Steneck and Johnson 2014) where they modify a range of abiotic factors including light, flow, sedimentation and scour (Kennelly 1989, Connell 2003, Toohey et al. 2004, Irving and Connell 2006). However, degradation of kelp forests including localised loss along sections of coastline and reductions in destiny and patch size have been observed in several regions (Steneck et al. 2002, Connell et al. 2008, Johnson et al. 2011, Moy and Christie 2012, Filbee-Dexter et al. 2016, Wernberg et al. 2016). Although a density reduction or loss of kelp are often suggested as being due to external factors such as ocean warming, increased sedimentation and overgrazing (Madsen et al. 2001, Ling 2008, Wernberg et al. 2016), the resilience of kelp populations may also depend on density-dependent modification of the abiotic environment which feeds back to positively influence kelp reproduction and recruitment. Density-dependent effects on kelp reproduction and/or recruitment can occur in two ways. First, a reduced density of adult sporophytes in a patch may reduce the net zoospore production and/or individual-level reproductive output. Second, zoospore density may determine gametophyte recruitment, fertilisation and thus sporophyte recruitment. As the dispersal of kelp zoospores is restricted by water motion (Gaylord et al. 2004) and the mobile antherozoids (sperm) can only detect pheromone omitted from non-motile eggs on female gametophytes within 1 mm (Boland et al. 1983), the density of zoospores and gametophytes is critical for fertilisation and thus, sporophyte recruitment (Reed 1990b, Reed et al. 1991). Kelps often have positive density-dependent vital rates (fertilisation, growth and reproduction; Schiel and Choat 1980, Schiel 1985, Reed

1990b, Reed et al. 1991) and high densities of visible macroscopic sporophyte recruits are observed beneath dense adult canopies (McConnico and Foster 2005).

Ecklonia radiata (hereafter *Ecklonia*) is the dominant ecosystem engineer of the Great Southern Reef (GSR) of southern Australia (Wernberg et al. 2005, Johnson et al. 2011, Bennett et al. 2016). This canopy-forming macroalga plays ecologically and economically important roles in this region providing food and habitat for diverse and productive food webs. For example, *Ecklonia* forests are important habitats for high value commercially important species such as abalone and rock lobster (Johnson et al. 2005, Johnson et al. 2011, Bennett et al. 2016). However, *Ecklonia* is under threat from increasing ocean temperatures (Mabin et al. 2013, Krumhansl et al. 2016, Vergés et al. 2016, Wernberg et al. 2016), increased sedimentation around urban areas (Gorgula and Connell 2004) and overgrazing by range-expanding sea urchins (Johnson et al. 2005, Ling et al. 2008, Ling et al. 2009). In particular, in south-eastern Australia the southern incursion of the East Australian Current has resulted in increases in ocean temps by >1°C since 1944 (Johnson et al. 2011) and these are predicted to continue to increase by 2.0 – 3.0°C over the next 100 yrs (Ridgway 2007).

Given the threats to *Ecklonia* and localised loss, it is important to understand how a decline in adult sporophyte density influences reproductive output, and how the density of zoospores ultimately influences early sporophyte recruitment and performance. In western Australia, a high cover of reproductive tissue and zoospore production occurs from mid-summer (January) to the end of autumn (May) and is positively correlated with seawater temperature (Mohring et al. 2013b). In contrast, in south-eastern Australia, *Ecklonia* attains a higher cover of sori during autumn and winter with almost no reproduction tissue present during summer (Sanderson 1990), but nothing is known of the seasonal patterns in zoospore release. In this

study we combined a field study with a lab culture experiment to determine; i) the effects of adult *Ecklonia* density and time on reproductive output (zoospore release); ii) the relationship between zoospore release and water temperature; 3) the effects of zoospore density on female gametophyte recruitment, sporophyte recruitment, and size at two times (winter and spring) and; 4) the relationship between early sporophyte recruit density and size at two times.

Materials and methods

Zoospore release

Monthly patterns of *Ecklonia* zoospore release was measured at Fortescue Bay (43.122898S, 147.974653E) in southeast Tasmania, Australia. Two cores (18-mm-diameter) of sori were sampled using a metal corer from the central lamina of 10 haphazardly selected reproductive adult thalli at an approximate depth of 10 m within a healthy *Ecklonia* forest. *Ecklonia* develops reproductive tissue along the central lamina and laterals (Kirkman 1981, Novaczek 1984, Mabin et al. 2013). Both cores from the same individual were placed in a small container (both sides of each core were reproductive resulting in a total surface area of 1017.9 mm^2 sori per individual) containing seawater and transported back to the lab. In the lab, the cores were placed on a clean paper towel and dried in a dark cool room (12 ˚C) for two hours and then, the cores from the same individual were soaked in 250 ml of 0.2 µm filtered seawater and kept under constant (133 µmol photon m^{-2} sec^{-1}) light in the cool room for approximately one hour. Samples were shaken well at the beginning and end to induce zoospore release and ensure adequate mixing. After one hour, the cores were removed, and the density of zoospores was determined using a haemocytometer. Zoospore density was converted to number of zoospores per mm^2 of surface area taking into account both surfaces of both sori for each individual. Measurements were taken every month from January 2012 to January 2013.

The effects of a reduction in adult *Ecklonia* density and time on zoospore release was determined by manipulating adult *Ecklonia* density in plots at Fortescue Bay. We established nine 5 m x 5 m plots at a depth of 11 m ± 2 m in a healthy and continuous *Ecklonia* forest. The nine plots were then allocated randomly to one of three density treatments (i.e. $n = 3$ replicates of each): low (1.48 ± 0.12 m^{-2}), medium (4.48 ± 0.25 m^{-2}) and high density of sporophytes. The 'high' density treatment comprised unmanipulated plots supported sporophytes at natural density (i.e. 9.3 ± 1.5 *Ecklonia* m^{-2} at the beginning of the experiment). The plots were separated from one another by at least 5 m and only the central 3 m x 3 m area (marked by ropes) was used to determine zoospore release to avoid edge effects. These densities were established in February 2014 and maintained for 24 months by removing adults as needed approximately every three months.

Zoospore release was determined 10 times over the 2-year period. Each time, one core of reproductive tissue was collected from five randomly selected adults within each plot using a 10-mm-diameter metal corer. In order to minimise thallus damage, only reproductive tissue on the laterals was sampled. Cores from the same plot were pooled and placed into a small jar underwater (total surface area of 785.4 mm^2 sori per plot). Back on the boat, the cores were removed from the water, dried with paper towel and placed into dry containers with more paper towel, and kept in a dark cool box for transport. On return to the lab, the samples were placed in the dark at a constant temperature (between 12 – 17 ˚C) overnight to dry. All cores from each plot were then placed into a small jar filled with 50 ml of sterilised 0.2 µm filtered seawater and placed under a constant light (133 µmol photon m^{-2} sec^{-1}) for approximately one hour. This process stimulated zoospore release, and zoospore densities were determined using the method outlined above and converted to the number of zoospores per mm^2 of surface area.

Zoospore release data were analysed using a one-factor Analysis of Variance (ANOVA: time) or a two-factor ANOVA (sporophyte density x time). Test assumptions were assessed using diagnostic plots of model residuals. Where transformation of data was indicated the transformation was determined from the peak in log-likelihood (λ) obtained from Box-Cox plots.

We related zoospore release to fluctuations in sea surface temperature (SST). Monthly day/night time average SST estimates were obtained from satellite remote sensing by the Australian Ocean Data Network (ADON) by Integrated Marine Observing System (IMOS). The data provided SST for 0.02 x 0.02 degree grid just outside the Fortescue Bay (43.12S, 148.00E). Due to data resolution, it was not possible to obtain SST data within the bay. We also obtained the daily average of seafloor temperature (SFT) from May 2015 to March 2016 using an *in-situ* Hobo temperature logger placed in one of the plots. The relationship between sea surface temperature and zoospore release was tested by a linear regression for the 2012 data describing monthly patterns and ANCOVA (factor = density, covariate = temperature) was performed for the 2014-16 data describing the effect of density and time. The ANCOVA revealed a significant density x temperature interaction ($F_{2,81} = 3.927$, $P = 0.024$), therefore separate linear regressions were run for each density. All analyses were conducted using R studio (Ver. 1.0.136) and the *MASS* package in R (ver. 3.4.4).

Effects of zoospore density on gametophyte and sporophyte recruitment

To determine the effects of initial zoospore density on female gametophyte and sporophyte recruitment and sporophyte performance, we set up zoospore dilution series experiments in November 2014 and June 2015. At both times, reproductive tissue was collected from 12 haphazardly selected adult *Ecklonia* at ~11 m from Fortescue Bay. The adult thalli sampled

were at the same site but outside of the experimental plots used to determine density effects on reproductive output. The sori were transported back to the lab in seawater and kept overnight with aeration at 17 ºC in November and at 12 ºC in June, representing approximate summer and winter temperatures respectively at the site. All sori were cut down to approximately the same size (50 x 100 mm) and washed in 500 ml of sterilised 0.2 µm filtered sea water containing 1% Betadine®. Sori were then rinsed in sterilised 0.2 µm filtered seawater to remove the Betadine®, placed between clean paper towels, and left in a dark cool room overnight. The clean sori were then placed in a sterilised container filled with one litre of sterilized f/2 sea water media (SWM; see Andersen 2005). The sori in the container were kept under a constant light (133 µmol m^{-2} sec^{-1}) for 1 hour to induce zoospore release. The resulting concentrations of zoospores were determined using a haemocytometer and were 341,250 ml^{-1} for November 2014 and 837,500 ml^{-1} for June 2015. For both experiments, there were 12 zoospore density treatments, with the highest density being the undiluted stock solution and the remainder of the treatments determined based on a $\log_2$ serial dilution. Culturing was done in 500 ml jars, each containing three cover slips: one for gametophytes, one for sporophytes, and a third one as a spare. The highest (undiluted) density treatment had 68.28 ml (in the November cohort) and 69.68 ml (in the June cohort) of zoospore stock solution (zoospores released in SWM) and the other zoospore density treatments were made by adding the appropriate amount of stock solution to jars containing 50 ml of UV sterilised SWM. Zoospore density mm^{-2} for each treatment was calculated from the total number of zoospores poured in a jar divided by the total horizontal surface area available for zoospore settlement (Table 4.1). Jars were kept in the dark for 48 hours to ensure zoospore settlement, then placed under light at 133 µmol photon m^{-2} sec^{-1} on a 12 h : 12 h, light dark cycle at either 17 ºC (November 2014) or 12 ºC (June 2015).

Table 4.1: Zoospore density ml^{-1} converted to density mm^{-2} on glass slides.

Treatment	November		June	
	ml^{-1}	mm^{-2}	ml^{-1}	mm^{-2}
1	0.18	0.0025	0.76	0.0106
2	0.67	0.0091	2.70	0.0374
3	2.50	0.0340	9.56	0.1326
4	9.30	0.1265	33.87	0.4695
5	34.60	0.4707	119.93	1.66
6	128.71	1.75	424.70	5.89
7	478.85	6.51	1503.94	20.85
8	1781.44	24.23	5325.77	73.83
9	6627.45	90.16	18859.61	261.44
10	24655.95	335.41	66785.70	925.81
11	91727.00	1247.83	236501.63	3278.48
12	341250.00	4642.29	837500.00	11609.76

After 14 days one coverslip was removed from each jar and 10 photos taken under a microscope (magnification = 100x) and the density of female gametophytes counted. After 40 days, a second cover slip was removed, and 10 photos taken under 40x magnification to determine the density and size of microscopic sporophytes. For the assessment of size, the surface area of 10 haphazardly selected sporophytes from each jar was measured using ImageJ 1.50. The density of female gametophytes, microscopic sporophytes, and the percentage transition from gametophytes to sporophytes were estimated. Sporophyte size was initially analysed using analysis of covariate (ANCOVA) with cohort (November 2014 and June 2015) as a fixed factor and sporophyte density as a covariate. Since the ANCOVA indicated dissimilar slopes ($P = 0.018$), the relationship between sporophyte density and size was then tested by individual regressions for each cohort.

Results

Zoospore release

Zoospore release in *Ecklonia* was dependent on the time of year ($F_{12,117} = 40.497$, $P < 0.001$). There were no zoospores released from sori sampled in January and February 2012. Although

there were some zoospores released (< 800 mm^{-2}) in October 2012, that amount was still significantly less than any other months (Tukey's test. $P < 0.05$, Fig. 4.1). The highest zoospore release was observed from April to August 2012 (excluding July) and in January 2013 (all of which were not significantly different from each other), with lower release rates in March and November 2012. In addition to October, the lowest non-zero release levels were recorded in July, September and December 2012, which were approximately 20% of that of the period of high zoospore release (Fig. 4.1).

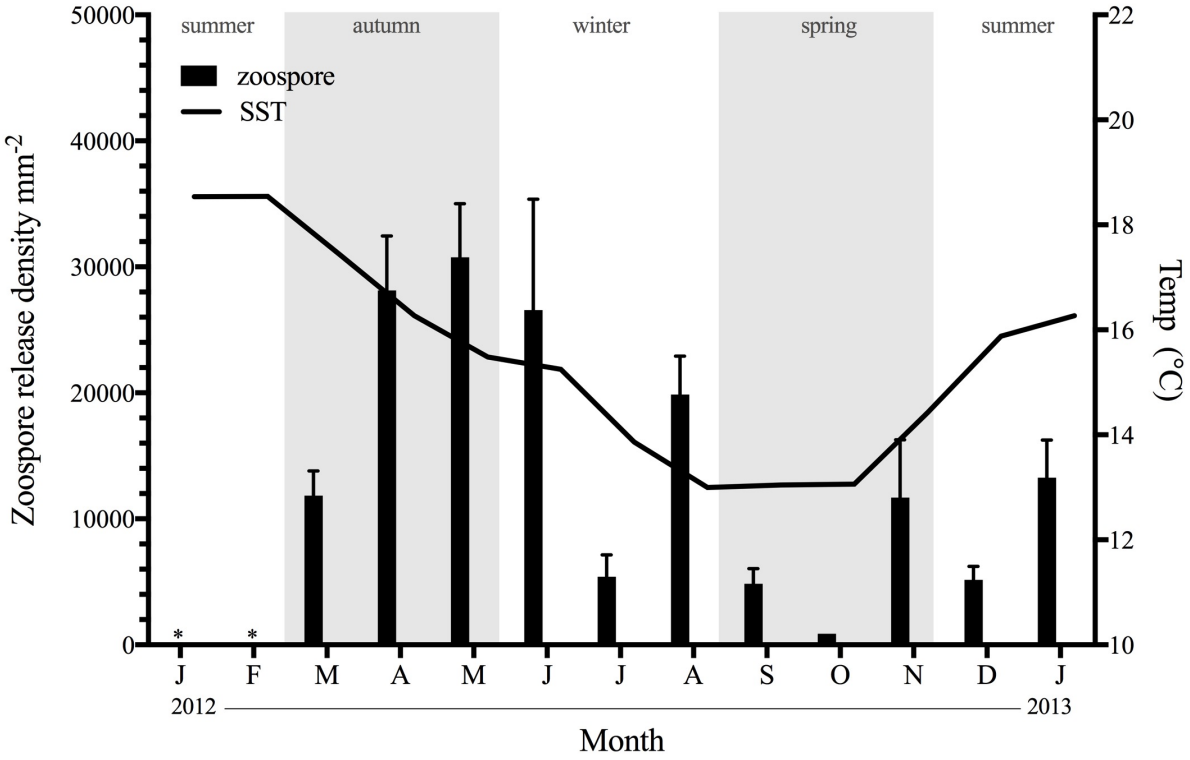

Figure 4.1: Seasonal pattern of zoospore release (mean ± SE) in *Ecklonia radiata*. January and February 2012 (marked with asterisks) released no zoospores. N = 10 thalli each time. SST = sea surface temperature (from satellite remote sensing data by Integrated Marine Observation System).

The effects of a reduction in sporophyte density and season on zoospore release indicated a significant interaction between density and time ($F_{2,18} = 2.663$, $P = 0.003$). This was driven by a single month (October 2015) which had very high zoospore release in the medium and high density treatments but significantly lower zoospore release in the low density treatment, and was the only month when there was a significant difference among density treatments

(Tukey's test, $P < 0.05$: [high = medium] > low). Although zoospore release changed significantly over time for each density, there was no clear seasonal pattern. Zoospore release was relatively high in March, July and September 2014 but then declined by almost 90% as SST increased with very few zoospores released in December 2014 (Fig. 4.2). Zoospore release increased again in May 2015 as the SST dropped but declined again in September 2015 when the water temperature was still low before increasing hugely in October 2015, especially in the high-density treatment when on average there was > 125, 000 zoospores released per mm^2 of sorus. At high adult density, zoospore release in October was significantly higher than all other times while zoospore release in December 2014 (159.2 ± 83.1 mm^{-1}, mean ± SE) was significantly lower than all other times (Tukeys tests, $P < 0.05$, Fig. 4.2). At a medium adult density, zoospore release in October was significantly higher than September 2015, April 2015, October 2014 and December 2014. Zoospore release in December 2014 was significantly lower than all other times (Tukeys tests, $P < 0.05$, Fig. 4.2). Zoospore release in the low density treatment were more consistent than high or medium, with no differences detected between March 2014, July 2014, September 2014 and from May 2015 – December 2015 (Tukeys tests, $P > 0.05$, Fig. 4.2). The months with the lowest zoospore release were October 2014, December 2014 and April 2015 which were all lower than in March 2014, July 2014, September 2014 and May 2015 (Tukeys tests, $P < 0.05$, Fig. 4.2).

There was a significant negative relationship between SST and zoospore release levels in plots with adult sporophytes at high (i.e. natural) density ($F_{1,27} = 8.225$, $P = 0.008$). Number of zoospores released from one mm^2 of sorus decreased by 12,421 ± 4,331 per 1 °C increase in SST (Fig. 4.3b). No significant relationships were observed for monthly sampling in 2012 ($F_{1,128} = 0.828$, $P = 0.365$, Fig. 4.3a), or from plots supporting adult sporophytes at medium

density ($F_{1,26}$ = 2.310, P = 0.141, Fig. 4.3c) and low density ($F_{1,28}$ = 0.007, P = 0.935, Fig. 4.3d).

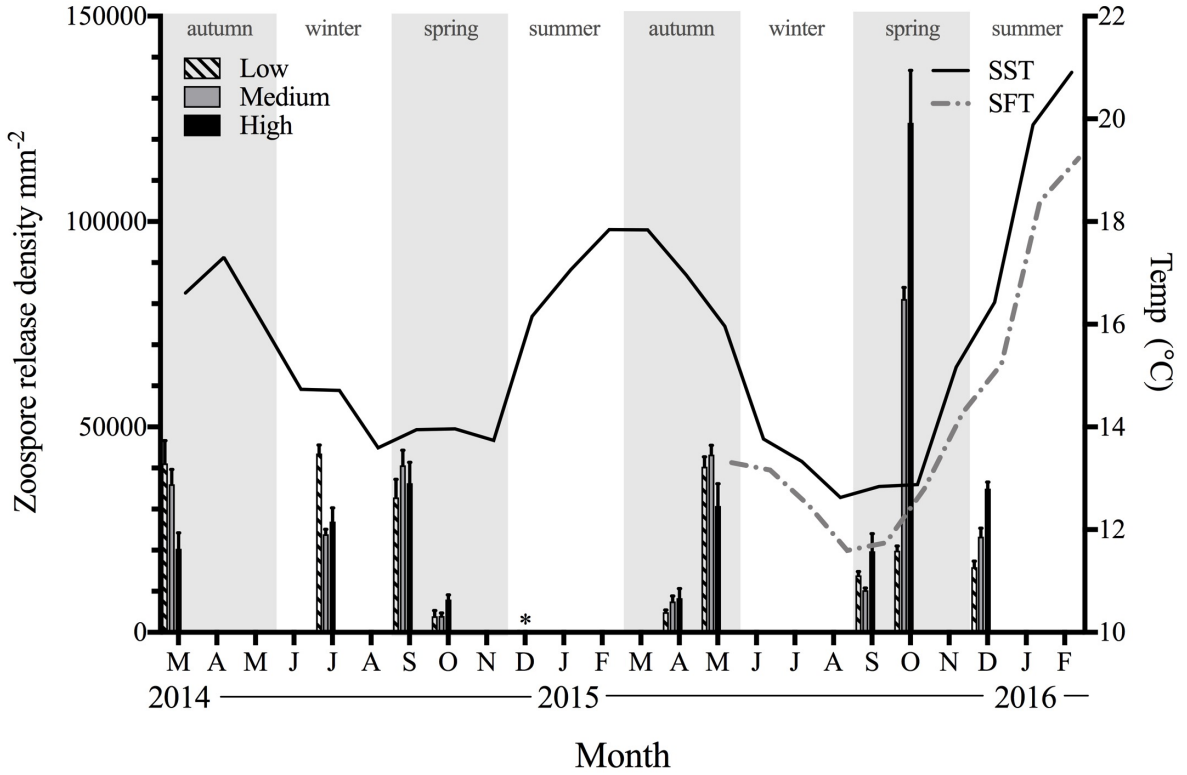

Figure 4.2: Effect of density of adult *Ecklonia radiata* sporophytes (Low = 1.48 ± 0.12, Medium = 4.48 ± 0.25, High = 9.3 ± 1.53, mean ± SE adults m^{-2}) in experimental plots, and season, on zoospore release (mean ± SE). In December 2014 (marked with an asterisk), thalli in the low density treatment released no zoospores while thalli in the medium and high density treatments released 159.2 ± 83.1 (mean ± SE) and are too small to be seen. N = 3 plots for each treatment each time. SST = sea surface temperature (from satellite remote sensing data by Integrated Marine Observation System), and SFT = sea floor temperature (determined from in situ loggers at the site).

Effects of zoospore density on gametophyte and sporophyte recruitment

The number of female gametophytes peaked at initial zoospore densities on the slides of between 260 – 1250 mm^{-2} for both November and June cohorts (Fig. 4.4a). Although both experiments showed similar hump-shaped relationships, for a similar initial zoospore density, in June a similar number of zoospores generally resulted in higher female gametophyte

densities. For both times, there was a decrease in female gametophyte density below an initial zoospore density of approx. 260 mm^{-2}, while at initial zoospore densities less than 70 mm^{-2} on the slides there were very few gametophytes, and virtually none were observed at less than two zoospores mm^{-2}. In the June cohort, the very high undiluted zoospore concentration (approximately 11,609 mm^{-2}) resulted in no gametophytes. For November, the high undiluted zoospore density (approximately 4,642 mm^{-2}) also resulted in lower female gametophyte densities (Fig. 4.4a).

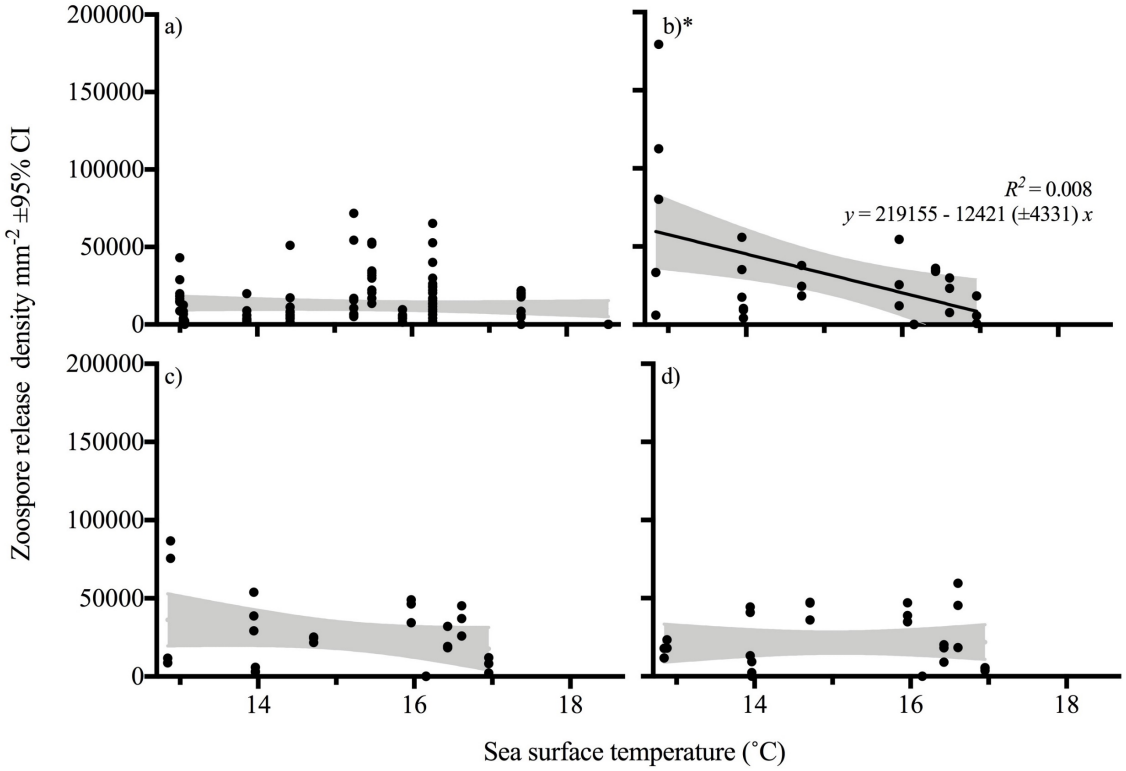

Figure 4.3: Linear regressions between sea surface temperature (SST) and mean *Ecklonia radiata* zoospore release a) from January 2012 – January 2013, and from March 2014 – December 2016 in b) high, c) medium and d) low adult *Ecklonia radiata* density treatments. The single significant relationship (for the high density treatment) is denoted with an asterisk.

Sporophyte densities developing on the glass slides also had similar hump-shaped relationships with initial zoospore density, but the optimal densities for each time were slightly different. There were very low sporophyte densities (<0.02 mm^{-2}) when initial zoospore density on the slides was low (< 6.52 mm^{-2}) and no sporophytes were found when it was less than 0.48 mm^{-2} in November, while we still found some sporophytes in June even

when initial zoospore densities were extremely low (< 0.47 mm^{-2}). At the other extreme, the June cohort developed no sporophytes when slides were incubated at very high initial zoospore densities (> 925 mm^{-2}).

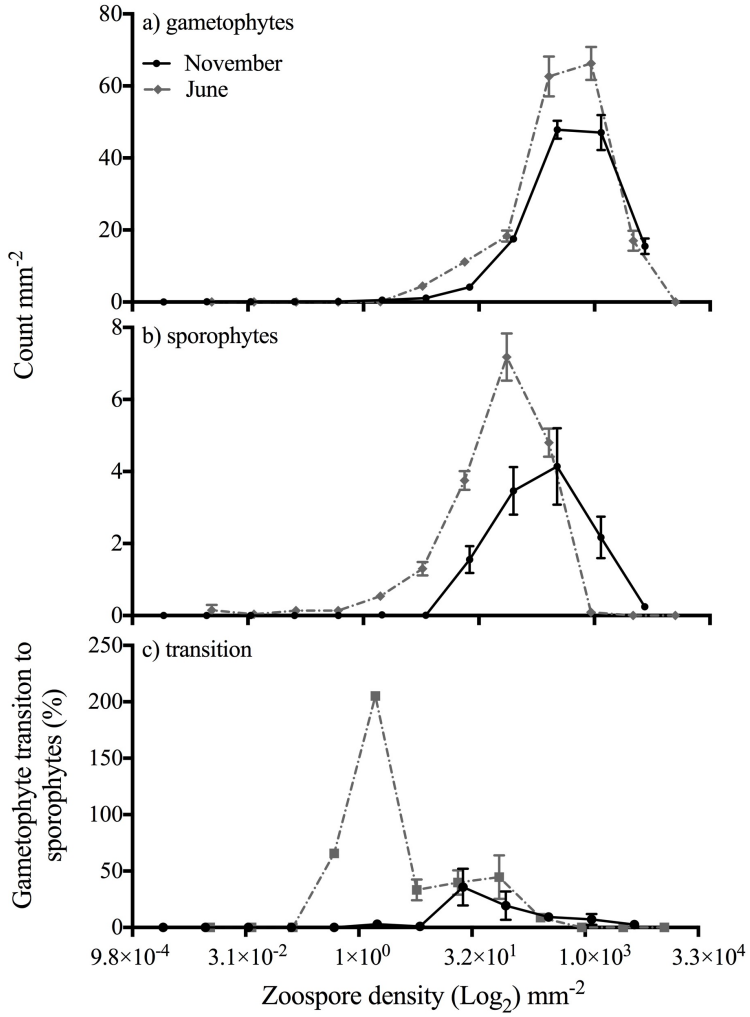

Figure 4.4: Effect of zoospore density in culture on the density (mean ± SE) of *Ecklonia radiata* a) female gametophytes settled to glass slides, b) resulting sporophytes, and c) the percentage transition (mean ± SE) between female gametophytes and sporophytes in spring and winter.

For November, the highest number of sporophytes occurred at an initial zoospore density of 335 mm^{-2} which also had the highest female gametophyte density. In comparison, in June the highest sporophyte density occurred at a lower initial zoospore density (74 mm^{-2}) than the highest female gametophytes density (initial zoospore density of 925 mm^{-2}). Interestingly, both cohorts had a similar number of female gametophytes either side of the peaks (initial

zoospore densities of 90 and 4642 mm^{-2} in spring and 74 and 3275 mm^{-2} in winter), but zero or almost no sporophytes recruited at higher gametophyte density at both times (Fig. 4.4b).

The transition from female gametophyte to sporophyte also varied with initial zoospore density, peaking at initial zoospore densities on slides of between 24 - 90 zoospores mm^{-2} (19 - 36% transition) in November and 0.5 – 74 zoospores mm^{-2} (~30% transition) in June (Fig. 4.4c). Notably at both times there appeared to be a threshold density for a significant transition from gametophytes to sporophytes to occur and this was lower in June (< 0.5 zoospores mm^{-2} compared to November: < 25 zoospores mm^{-2}). The observed high transition rate (~ 200%) in June with an initial zoospore density of 1.66 mm^{-2} was due to the low density of gametophytes after 14 days (0.1 ± 0.1 mm^{-2}) which resulted in a large chance of sampling error in calculating this transition (i.e. not detecting small gametophytes at 14 days but detecting larger sporophytes at 40 days).

Effects of sporophyte density on size

The density of sporophytes on the slides affected sporophyte size differently depending on time of the year ($F_{1,336} = 17.767$, $P < 0.001$). Sporophytes in the June cohort were much larger than those in the November cohort, and the magnitude of difference decreased with increasing sporophyte density. Sporophyte size in June declined with sporophyte density ($F_{1,19} = 7.377$, $P = 0.0137$) but no effect of density on size was observed in the November cohort ($F_{1,12} = 4.628$, $P = 0.0525$, Fig. 4.5).

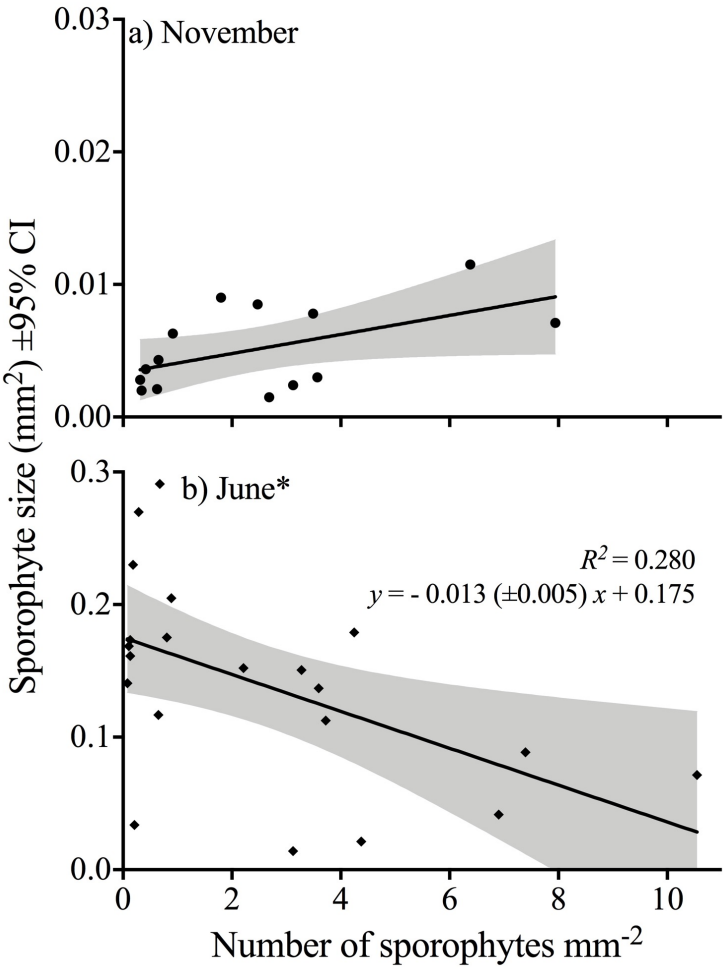

Figure 4.5: Linear regressions between mean *Ecklonia radiata* micro-sporophyte density and size cultured in a) November (spring) 2014, and b) June (winter) 2015 with the relationship only significant in June.

Discussion

This study has identified that zoospore production (number of zoospores released per area of sorus) in *Ecklonia radiata* fluctuates significantly throughout the year with evidence of a peak from mid-autumn to early-winter and that low zoospore production was more likely in summer. However, zoospore release per unit area of sorus was not strongly affected by adult sporophyte density, indicating that at any given time a higher density of reproductive adults in a kelp forest should result in an increased concentration of zoospores in that area. We also found a weak negative relationship between temperature and zoospore release in plots at high

sporophyte densities, but no such effects were observed at low or medium densities or in a natural kelp forest sampled in 2012. Because we observed that a minimum initial zoospore density was required for gametophyte and sporophyte recruitment, and there was an optimal gametophyte density (and thus zoospore concentration) for maximum recruitment, a decline in the local density of reproductive adult sporophytes resulting in fewer zoospores being released may lead to a decline in sporophyte recruitment.

Effects of adult density, season and temperature on zoospore release

Ecklonia has a large latitudinal and longitudinal distribution (Womersley 1981), and the time of peak reproduction appears to vary across regions. On the west coast of mainland Australia, high zoospore release is observed in late summer to autumn, and both the cover of sorus tissue and the number of zoospores released are positively related to temperature (Mohring et al. 2013a, Mohring et al. 2013b). This study shows that in Tasmania, a peak in zoospore release during autumn and winter coincides with falling seawater temperature and is lowest in summer when ocean temperature is highest. Our result is similar to previous findings of larger amounts of sorus tissue per sporophyte in cooler months in Tasmania (Sanderson 1990, Mabin et al. 2013). This onset of a peak in reproduction is similar to findings for the annual kelp *Undaria pinnitifada* in California, which released more zoospores following a decrease in temperature (Thornber et al. 2004). Similarly, *Ecklonia maxima* in South Africa have less reproductive tissue and release fewer zoospores in late summer with a peak in spring (Joska and Bolton 1987). The slight, but significant negative relationship between temperature and zoospore production for *Ecklonia* in the high density treatment in eastern Tasmania is opposite to the results for *Ecklonia* on the Western Australian coast. The overall temperature regimes in the different regions may influence the timing of peak reproduction; temperatures on the Western Australian coast at sites sampled by (Mohring et al. 2013b) ranged from 15 –

26 °C, which is notably warmer than the annual range at our site in Tasmania which is from 12 – 19 °C. In general, early life-cycle stages of *Ecklonia* appear to have a higher thermal tolerance than many kelps, with gametophytes viable and having high growth up to 22 °C (tom Dieck 1993, Mabin et al. 2013, Mohring et al. 2014).

Although the relationships between temperature and zoospore release in both western Australia and eastern Tasmania (only at natural 'high' density) were significant, they were relatively weak (low R^2 values, Mohring et al. 2013b) suggesting that other factors are important in explaining the variation in zoospore release. In kelp, day length and light levels are often cues for reproduction (Bolton and Levitt 1985, Nelson 2005) and the peak in reproduction in eastern Tasmania in April-June may be a response to a decrease in day length and/or light. Water motion is also known to influence zoospore release in kelp and in Western Australia, *Ecklonia* tends to release more zoospores when seasonal swells are low (Mohring et al. 2013b). In south-eastern Tasmania, there are spring/early summer blooms of ephemeral benthic algae (Graham 2004) which potentially compete with early life-cycle stages of kelps (Tatsumi and Wright 2016), so with a peak in reproduction at other times, *Ecklonia* in this region may avoid this competition. In general, the extremely high zoospore release we observed in October 2015 highlights the likely role of a number of factors affecting zoospore production. Nonetheless, the different zoospore production for *Ecklonia* in different regions likely reflects adaptations to different environmental conditions.

Given zoospore release appears at least partially related to temperature, results of the present study suggest that per capita zoospore production of *Ecklonia* in Tasmania may decrease with ocean warming. There has already been > 1˚C increase in average annual temperature since 1944 in the vicinity of Maria Island in eastern Tasmania (Johnson et al. 2011), and ocean

temperatures are predicted to keep increasing (IPCC 2014). The capacity for *Ecklonia* in Tasmania to adapt to increasing ocean temperatures will depend in part on the heritability of traits relating to thermal tolerance, but zoospore release will be reduced with increased SST and if adaptation for key reproductive and developmental traits is not fast enough, *Ecklonia* in Tasmania may not be able to produce sufficient numbers of zoospores to maintain recruitment.

The weak effects of density of adult sporophytes on zoospore release suggest that changes in environmental conditions following a decline in density do not have a major effect on reproductive capacity of individual thalli. The most consistent abiotic change associated with a decline in density is an increase in light (Layton et al. in review, Chapter 2). In contrast to our finding, Edwards and Konar (2012) found increased zoospore production in *Eualaria fistulosa* where they are exposed to higher light (urchin barrens) than lower light (heterospecific kelp forest). In *Ecklonia*, increased light typically has positive effects on growth of juveniles which is often reflected in the rapid post-recruitment growth observed following canopy loss (Kendrick et al. 2004, Toohey and Kendrick 2007, Flukes et al. 2014). At the depth of our sampling, light levels were around 200 μmol sec^{-1} m^{-1} (Chapter 2) in late spring at the benthos when there was no canopy which do not appear deleterious for mature or juvenile *Ecklonia*. However, a reduction in the density of reproductive adults should reduce zoospore concentration in the water column as the absolute number of zoospores released will decrease with as the density of reproductive adults declines. Shelamoff et al. (unpubl.) found that the number of *Ecklonia* recruits increased with the density of adults on reproductively isolated reefs. Even though per capita reproductive capacity is little affected by the density of adult sporophytes, population level output is likely to decrease with decreasing density of adult *Ecklonia*.

Effects of zoospore density on gametophyte and sporophyte recruitment

The relationships between zoospore density and both gametophyte and sporophyte densities were non-linear, and highlight critical minimum and optimal zoospore densities for sporophyte recruitment. The minimum densities for successful sporophyte recruitment were lower in winter (June) compared to late Spring (November) and for a given zoospore density, sporophyte recruitment was higher in winter until very high sporophyte densities (> 260 zoospores mm^{-2}) were reached. The reasons for the higher sporophyte recruitment in winter at a similar zoospore density may relate to temperature and/or the quality of zoospores at the time of collection. Given that early life-cycle stages of *Ecklonia* are thermally tolerant and sporophytes can develop at temperatures above 22.5˚C (tom Dieck 1993, Mabin et al. 2013, Mohring et al. 2014), the differences in temperature between our experiments (12°C in June and 17°C in November) is unlikely to have been crucial, but may have had a slight effect the generally higher recruitment in winter. Kelp zoospores contain lipids that, along with photosynthesis, provide energy during swimming and germination (Brzezinski et al. 1993, Reed et al. 1999). The lipid content increases with temperature to a point, but then decreases above the optimal temperature (Converti et al. 2009). Our culturing method had a 48 h dark period immediately following zoospore release into the culturing jars. As lipid decreases significantly under no photosynthesis (Reed et al. 1999), together with temperature difference between the cohorts, this might affect mobility of zoospores and result in a requirement for a higher zoospore density for optimal germination in our November cohort. The result of zero gametophytes (at zoospore density > 11609 mm^{-2}) and sporophytes (zoospore density > 925 mm^{-2}) in the June cohort maybe a result of 'hyper densities' of zoospores accelerating intraspecific competition for space, nutrients, or other resources (e.g. oxygen).

Virtually no sporophytes recruited when the zoospore densities were less than 1.7 mm^{-2} and very low sporophyte number (< 0.02 mm^{-2}) when the zoospore densities were < 24 mm^{-2} in November, and less than 0.54 sporophyte mm^{-2} when zoospore densities were < 5.8 mm^{-2} in June. At low gametophyte densities, males and females may not be sufficiently close to each other for fertilisation to occur. In the field, minimum zoospore densities of 1 mm^{-2} have been identified as a critical threshold for successful fertilisation and sporophyte development in *Macrocystis pyrifera* and *Pterygophora californica* (Reed 1990a, Reed et al. 1991). The finding that these minimum densities for *Ecklonia* differ over time further highlighted possible temporal effects on zoospore, gametophyte, and gamete quality. The optimal zoospore density for sporophyte recruitment occurred between approximately 20 to 335 zoospores mm^{-2} at both times. The absence of a temporal effect for these optima indicates that once a high enough density is reached then factors affecting zoospores, gametophytes, and gamete quality may become less critical than they are at lower densities. However, it is clear that there is also an upper threshold of zoospore density (> 925 zoospore mm^{-2} in June and 4,642 mm^{-2} in November) at which no sporophytes develop, suggesting negative density-dependent effects at early life-cycle stages.

The finding that a minimum zoospore density is required for sporophyte recruitment highlights the importance of maintaining a minimum density of adult sporophytes for zoospore supply (Reed 1990a, Schiel and Foster 2006). The concentration of *M. pyrifera* zoospores in the water column is strongly coupled to local reproductive output (Graham 2003) indicating that healthy, high-density kelp forests are likely to produce more zoospores per unit area of benthos compared to lower density forests. However, we do not know the concentrations of *Ecklonia* zoospores in the water column or densities at settlement. For *M. pyrifera*, zoospore concentrations measured in the water column ranged from 250 – 54, 000

zoospores/L but were usually less than 5, 000 zoospores/L (Graham 2003). Clearly, a critical zoospore concentration beneath the canopy is necessary to realise adequate *Ecklonia* sporophyte recruitment. Given that 1) zoospore production per mm^2 sorus was not affected by adult density, 2) to the best of our knowledge, the sorus area on individual thalli is also not affected by adult density, and, 3) most zoospores will not disperse more than a few 10s of metres, zoospore concentration within *Ecklonia* forests must be dependent on local adult density. Although there is no evidence for reduced flow beneath the *Ecklonia* canopy (Wernberg et al. 2005, Chapter 2) that might help to retain zoospores, the reduced light beneath a full canopy is likely to have positive effects on zoospore settlement and gametophyte recruitment. Kelp zoospores are active under high irradiance and tend to settle in darker, shaded areas (Reed et al. 1992) such as under a full adult canopy. Moreover, low light conditions enhance *Ecklonia* gametophyte recruitment and also reduce understory algal abundance (Toohey and Kendrick 2008, Tatsumi and Wright 2016). With a full canopy, the combination of high zoospore production and low light may provide the conditions for zoospores to aggregate and thus, maximise recruitment. A high concentration of zoospores may also contribute to localised expansion of kelp forests. Long-distance zoospore dispersal depends on water movement and the concentration of zoospores decreases with distance from the source population (Reed et al. 1992, Cie and Edwards 2011).

We observed a negative relationship between sporophyte density and size on glass slides in June (winter), but not in November (spring). Also, the sporophytes in the winter were much larger than those of similar age in spring, which we interpret as evidence of intraspecific competition among the sporophytes in winter. Although higher growth of algae is commonly observed during warmer months, it is likely to be due to higher light availability (Miller et al. 2011, Tatsumi and Wright 2016). Bearham et al. (2013) found that an increase in temperature

negatively influences *Ecklonia* growth. Given our experiment was conducted under the same light for both cohorts, smaller sporophyte size in November may be due to higher temperature. Biomass-density relationship in plants and seaweed have been long recognised (Schiel and Choat 1980, Scrosati 2005). A high density of adult *Ecklonia* ensures higher zoospore concentration, therefore high sporophyte recruitment. Although a high sporophyte density can have negative effects on growth rates and thus size, elimination of slow growing individuals via self-thinning may be part of the recruitment cycle selecting for fitter individuals.

Conclusion

Overall, our study indicates that there is large temporal variation in zoospore release by *Ecklonia radiata*, but zoospore release per unit area of sorus is not affected by the density of adult sporophytes. Sea surface temperature only explained very limited, but significant, variation in zoospore release at natural sporophyte density. We also identified strong density-dependent effects of zoospores on subsequent recruitment of sporophytes, including minimum and optimal zoospore densities for sporophyte recruitment, which also changed with time. The net conclusion of these findings is that a declining density of adult *Ecklonia* is unlikely to affect per capita zoospore production but is likely to result in a population-level decline in zoospore concentration which will ultimately decrease recruitment and influence the resilience of *Ecklonia* forests. Further study on zoospore density in the field at different densities of adult sporophytes is required to increase our understanding of the importance of thresholds in zoospore density necessary to maintain healthy kelp forests.

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Chapter 5. General discussion

Threats to ecosystem engineering kelp

Ecosystem engineers play critical roles in many systems by modifying abiotic and biotic resources (Jones et al. 1994, Coleman and Williams 2002, Hastings et al. 2007, Jones et al. 2010). Habitat forming ecosystem engineers, such as kelps, engineer environments via structural modification (e.g. holdfast, stipe and canopy) which causes abiotic and biotic change. The net effect is the creation of a complex habitat that enhances species diversity and productivity and these facilitative effects can be far-reaching with entire communities dependent on the presence of the engineers (Silliman et al. 2011, Byers et al. 2012). The common kelp, *Ecklonia radiata* (herein *Ecklonia*), is the dominant subtidal ecosystem engineer of the Great Southern Reef (GSR) of Australia (Wernberg et al. 2005, Johnson et al. 2011, Bennett et al. 2016). *Ecklonia* forests support diverse assemblages of ecologically and economically important species such as *Haliotis rubra*, *H. laevigata* and *Jasus edwardsii* (Johnson et al. 2005, Johnson et al. 2011, Bennett et al. 2016). The economic value of commercial and recreational fishing in the GSR is well over AU$1,000 million per year and it contains 1,499 seaweed species, 4100 invertebrates and 731 fishes (Wernberg et al. 2013b, Bennett et al. 2016). A large part of this highly diverse and productive system is underpinned by trophic resources and environmental modifications provided by *Ecklonia* (Wernberg et al. 2013a, Bennett et al. 2016). Much research has focused on the effects of structural, abiotic and biotic changes caused by *Ecklonia* on associated species (Kendrick et al. 1999, Connell 2003, Toohey et al. 2004, Flukes et al. 2014, Wernberg et al. 2016, Coleman and Wernberg 2017), and less is known about how those changes might feedback to affect its own demography. These "environment-engineer feedbacks" will occur when the demographic rates of an engineer are themselves affected by the engineered environment (Jones et al.

2010). Thus, degradation of populations of *Ecklonia* may have negative implications due to a decline or loss of feedback effects.

The degradation of kelp forests, due to climate change and increased anthropogenic stressors, has been reported world-wide (Jackson et al. 2001, Airoldi 2003, Ling 2008, Krumhansl et al. 2016, Wernberg et al. 2016). On the eastern and western coastlines of Australia, ocean warming and the southward migration of warm water grazers are reported as major drivers contributing to range contractions and declines in the density of kelp forests (Smale and Wernberg 2013, Wernberg et al. 2013a, Vergés et al. 2014, Wernberg et al. 2016). For example, in 2011 an extensive marine heatwave on the west coast of Australia significantly reduced the biomass of *Ecklonia* and shifted the system to turf algae dominated at the northern end of *Ecklonia* distribution (Wernberg et al. 2013a). On the east coast of Tasmania, the southwards incursion of the East Australian Current (EAC) has resulted in > 1 ˚C increase in sea surface temperature since 1944 and is expected to increase by 2.0 – 3.0 ˚C over next 100 yrs, four times higher than the expected global average (Cai et al. 2005, Ridgway 2007, Johnson et al. 2011). The EAC has also contributed to range expansion of the barrens forming sea urchin, *Centrostepahnus rodgersii,* which were only found on mainland Australia until the 1960s, but are now abundant in Tasmania (Johnson et al. 2005, Ling and Johnson 2012). These examples highlight the breadth of research focussing on external drivers of kelp forest degradation, but internal drivers, such as how a decline in density might influence environmental feedbacks to *Ecklonia* demography is unclear. Therefore, I aimed to determine; (i) how a decline in the density of *Ecklonia* affected engineering of critical abiotic factors and the link between those abiotic changes and both the understory community and *Ecklonia* reproduction, recruitment and post-recruitment growth and survivorship, (ii) whether abiotic factors modified by *Ecklonia* were important mechanisms affecting the early post-recruitment survivorship and growth of *Ecklonia* sporophytes, and; (iii) how density-

dependent effects on reproductive output affected recruitment of both gametophytes and sporophytes.

Effects of a reduction in kelp density on the abiotic environment, understory algae and kelp recruitment

The availability of sub-canopy light has been long noted as an important abiotic factor determining sub-canopy communities and success of *Ecklonia* recruits (Novaczek 1984, Dayton 1985, Kennelly 1987, Toohey and Kendrick 2008, Flukes et al. 2014). My study identified strong density-dependent light reduction (Chapter 2) and average photosynthetic active radiation (PAR) did not exceed 20 µmol photon m^{-2} s^{-1} under a full (natural) canopy. Kelp zoospores are more active under light, but tend to settle in darker, shaded areas (Reed et al. 1992) and thus *Ecklonia* zoospores may be more likely to settle under a full canopy. In addition, *Ecklonia* gametophytes recruit at higher densities under low light (PAR < 10 µmol photon m^{-2} s^{-1}) but moderate light (at least 40 µmol photon m^{-2} s^{-1}) results in higher growth and the production of sporophytes (Novaczek 1984, Tatsumi and Wright 2016). Similarly, Graham (1996) found that gametophytes of *Macrocystis prifera* are negatively influenced by excessive light, that is gametophyte success increases with depth (lower light). Kelp gametophytes are able to survive and stay fertile under such low light conditions for extended periods of time until adequate light is available after which gametogenesis and the subsequent development of sporophytes occurs (Novaczek 1984, Edwards 2000). A higher PAR will also increase the biomass of understory algae (Kendrick et al. 1999, Toohey 2007, Tatsumi and Wright 2016) and as understory algae can significantly reduce the survivorship of kelp recruits (Tatsumi and Wright 2016), this may be an indirect mechanism affecting sporophyte recruitment. Overall, this evidence suggests that light reduction by a full *Ecklonia* canopy supports early recruitment of *Ecklonia* by reducing abiotic and biotic stresses.

My experiment looking at the interactive effects of abiotic factors on microscopic sporophytes (Chapter 3) showed that sporophytes survive better under low light than ambient light (in the absence of scour with ambient water flow) after six weeks of outplanting. This lower density under ambient light may be due to photoinhibition or intraspecific competition. Young sporophytes are more susceptible to photoinhibition, becoming more robust as they grow, although photoinhibition in juvenile kelps is usually observed in shallow areas where PAR exceeds 500 µmol photon m^{-2} s^{-1} (Hanelt et al. 1997, Altamirano et al. 2004). PAR during this experiment never exceeded 250 µmol photon m^{-2} s^{-1} and if ambient light caused any harm to recruit, it should have been more prominent in week three than in six. The lower survivorship under ambient light in week six may instead indicate early intraspecific competition. Sporophytes after six weeks under ambient light were approximately 2.8 times larger than those under low light. Such competition may increase resilience of *Ecklonia* forests via a selection of fast-growing individuals for when adequate light is received, allowing the canopy to reform before understory algae increase in abundance. Rapid reformation of kelp canopies after canopy clearance is often observed in healthy kelp forests (Kennelly 1987, Dayton et al. 1992, O'Connor and Anderson 2010). The transplanted stage 1 juveniles exposed to high light under the full canopy removal (zero density treatment, Chapter 2) had a significantly higher growth rate than any of the other treatments with adult *Ecklonia* in the plots. Thus, the likelihood of a higher density of zoospores providing a 'seed bank' of gametophytes and/or microscopic sporophytes under a high density of reproductive sporophytes, combined with rapid growth of sporophytes on exposure to high light following disturbance, may underpin the rapid recovery of the canopy. In addition, *Ecklonia* occurs in much shallower areas where PAR exceeds the fatal level (Novaczek 1984, Bennett et al. 2016) and in those situations, the adult canopy may provide a stronger benefit to juveniles.

Benthic scour decreased as the density of adult stipes increased which contradicts with the prediction of higher canopy abrasion from scour with increasing *Ecklonia* density (Connell 2003). *Ecklonia* has large morphological variation and as the *Ecklonia* canopy in Tasmania does not lay near the benthos, the observed decrease in scour with increased density is likely to be due to dense thalli blocking the movement of neighbouring thalli. Scour had strong effects on the survivorship of very small sporophytes with mortality at least 50% higher in the presence of scour. Although sporophytes appear to be more resilient towards scouring as they grow larger (Chapter 3), microsites beneath the canopy that are safe from scour appear crucial for the survivorship of small *Ecklonia* sporophytes. Anderson et al. (1997) found higher aggregations of juvenile *Ecklonia maxima* on its adult holdfasts where they are less likely to be exposed to canopy scour. The outplanting of microscopic sporophytes into different densities of *Ecklonia* (Chapter 2) showed low survivorship in both June (winter) and November (late spring) 2014. In June, I found hardly any surviving sporophytes after 42 days of outplanting but the highest survivorship was in the high adult-density treatment. My lab experiment showed higher sporophytes densities in winter than spring (Chapter 4), therefore, the observed extremely low survivorships across all treatment in the field in winter may have been caused by lower light in winter or, extreme events, such as winter storms that resulted in greater scour. Higher survivorship was observed in November compared to June. Although it was not statistically significant, there was a trend for survivorship to be lower as adult density increased. Scour rate under intact canopy was significantly lower in this study, but as the outplanting racks were elevated 30 cm above the substrate, to avoid benthic grazing, this might have increased scour from the adult canopy. Overall, the low light, low scour conditions which occurs at a high adult density appears the best environment for the survivorship of microscopic stages.

The depth of accumulated sediment in the turf was reduced by the presence of adult *Ecklonia* and although we could not identify a significant difference between the high, medium and low kelp density treatments, there was a trend for it decreasing with increasing adult density (Chapter 2). Sediment accumulation on the benthos has been suggested as being the result of limited canopy sweeping (Kennelly 1989) and our findings for lower sediment in the presence of *Ecklonia* were supportive of that. This study also found no effect of density on the rate of sub-canopy sediment deposition, that is deposition is determined by the amount of suspended sediment in a water column. This indicates that canopy scour is not the primary factor determining sediment accumulation. Higher benthic sediment in areas dominated by turf algae is often observed after loss of *Ecklonia* (Wernberg et al. 2016). Turf algae trap sediment particles creating a turf sediment matrix (Birrell et al. 2005). The attachment of kelp zoospores and gametophytes are significantly reduced by increased sediment loads (Arakawa 2005, Geange et al. 2014, Watanabe et al. 2016). I initially planned to test interactive effects of sediment loads with other abiotic factors on microscopic sporophytes (Chapter 3), but it was not possible as there was no effective way to maintain realistic sediment loads in the field.

Despite no significant effects of density treatments on the number of natural recruits, I observed blooms of recruits in manipulated treatments up to seven months post-adult manipulation (Chapter 2). Following this, the number of recruits in these plots decreased and remained low. This result is in line with other studies which also showed high recruitment after canopy removal in kelp (Toohey and Kendrick 2007, O'Connor and Anderson 2010, Flukes et al. 2014) and likely reflects the existence of a sub-canopy "seed-bank" of microscopic stages. Low recruitment after the initial post-manipulation bloom may be an indication of a decline of positive engineering feedback effects. Survivorship of stage 1

juveniles was not affected by adult density and some of transplanted juveniles into the experiment plots grew to stage 2 to 3 forms (see Kirkman 1981) with over 430 mm in height in less than seven months in all treatments, except in the high density treatment (Chapter 2). As the reduced density treatments were maintained for two years, newly grown canopy-forming adults were removed as required preventing canopy recovery. This kept higher sub-canopy light high which may have enhanced the growth of understory algae (Kennelly 1989, Clark et al. 2004, Flukes et al. 2014). Although I did not identify a strong change in understory algae composition, biomass was significantly higher when there was no *Ecklonia* compared to high density of *Ecklonia*, and there was a trend for low and medium densities to have higher biomass than the high density treatment. Higher sediment accumulation and understory algal biomass with no *Ecklonia* canopy may have negatively affected recruitment success.

One of the important findings from the long-term field experiment was that many of our abiotic measurements had large variation within the same density treatment which may have swamped any density effects. This highlights the importance of microscale habitat variation within kelp forests. Our measurements of kelp demography (survivorship, height, growth and erosion) also showed large within-treatment variation. Topographical complexity can cause variation in the abiotic environment by affecting downwelling light and water motion (Toohey 2007). Complex reef structures may also cause a change in canopy scour as the distance from the canopy to reef surfaces changes. Such variations in the abiotic environment due to complex topography can also increase the diversity of algal assemblages as there are more microhabitats available for different algal species (Toohey 2007, Toohey et al. 2007).

Density-dependent reproduction and sporophyte production

Successful microscopic sporophyte recruitment depended on the initial density of zoospores and there was a minimum threshold zoospore density (< 1.6 in winter and < 6.5 mm^{-2} in spring), and optimal zoospore density (ranged from 20 – 260 in winter and 74 – 335 mm^{-2} in spring, Chapter 4). I also observed large temporal variability in zoospore release in the field and limited evidence of adult density affecting zoospore production. To the best of my knowledge, there is no density dependent reduction of sori area on a thallus, suggesting that net zoospore production should increase as adult density increases. Zoospore concentration in a water column in a natural *Ecklonia* forest is not known although at a gross level, the number of zoospores released from a given surface area of sori may be an adequate approximation. Graham (2003) measured zoospore concentration of *Macrocystis pyrifera* in a water column (3 cm above the benthos) and found that zoospore concentration ranged from 250 to over 54,000 zoospore per litter of water which would equate to a benthic zoospore density of 0.025 to 5.4 mm^{-2} if they settle equally. Although zoospore production in *Ecklonia* can be higher and lower, it seems unlikely that zoospore density in the field would exceed the higher end of optimal zoospore density (> 335 mm^{-2}) where negative effects were observed in the lab. Given kelp zoospores are more active under light and tend to settle in dark shaded areas (Reed et al. 1992) zoospore density and microscopic sporophyte recruitment may be higher beneath lower light canopies. Although only a partial negative effect of increased sea surface temperature on zoospore release was observed, predicted ocean warming my further influence zoospore production in Tasmania population.

Conclusion

This study identified that some abiotic factors change in response to a decline in adult *Ecklonia* density and good recruitment conditions (low light, low scour with ambient flow) are more likely to be found more in dense forests. Although the demographic responses of microscopic and macroscopic juveniles to these changes were not strong, a high adult *Ecklonia* density should result in a higher zoospore concentration and thus sporophyte recruitment, and the low light, low scour conditions should increase the survivorship of microscopic sporophytes. A gap in the canopy would then enhances their growth which is likely to be important for reformation of the canopy after disturbance. Knowledge gained from this study on the preferred abiotic conditions for microscopic sporophyte survivorship can assist in the restoration and recovery of *Ecklonia* forests. Nonetheless, further research is needed to understand how the demographic importance of the 'seed-bank' and its contribution to the resilience of *Ecklonia* forests.

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